

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
MOLINATE (ORDRAM)

Chemical Code # 000449, Tolerance # 00228
SB 950 # 208

April 7, 1987

Revised 6/17/88, 7/27/89, 12/19/89, 1/25/90, 4/10/90, 5/9/90, 7/26/90, 4/29/91, 8/01/91, 10/15/91,
3/13/92, 5/4/92, 6/30/94, 8/11/94, 5/10/95, 7/11/96, 1/16/97, 7/8/98, 11/19/98, 12/30/99, and
9/6/00

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, possible adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	There is no acceptable hen study, but there are acceptable rat studies (acute, subchronic, and developmental neurotoxicity studies).

Toxicology one-liners are attached. Note: these pages contain summaries only. Individual worksheets may identify additional effects.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

Revised by G. Chernoff 7/26/90, and by C. Aldous, 4/29/91, 8/01/91, 10/15/91, 3/13/92, 5/4/92, and 6/30/94; Gee, 8/11/94; Aldous, 5/10/95 and 7/11/96; by Gee, 1/16/97, by Aldous, 7/8/98, 11/19/98, 12/29/99 and 9/6/00.

All relevant records on file with DPR as of 9/01/00 have been included in the Toxicology Summary. These include record numbers through 176747 (in Document No. 228-189). Also, there are older record numbers of the 900000+ series.

TABLE OF CONTENTS

COMBINED, RAT	3
CHRONIC TOXICITY, RAT	4
CHRONIC TOXICITY, DOG.....	4
CHRONIC TOXICITY, MOUSE.....	5
ONCOGENICITY, MOUSE.....	5
REPRODUCTION, GENERAL COMMENTS	6
REPRODUCTION, RAT.....	7
Rat Multi-generation Studies: Both Sexes Treated	7
Rat Reproduction, Crossover Studies.....	9
Rat Reproduction, Treatment Restricted to Females	9
Rat Reproduction, Treatment Restricted to Males (oral route only)	11
Rat Reproduction, Treatment Restricted to Males (inhalation route)	14
Rat Reproduction, Related Studies, Acute to Subchronic	15
REPRODUCTION, MOUSE.....	17
REPRODUCTION, MONKEY.....	18
REPRODUCTION, RABBIT.....	19
REPRODUCTION, HUMAN EPIDEMIOLOGICAL STUDIES	22
TERATOLOGY, RAT	24
TERATOLOGY, RABBIT	24
TERATOLOGY, MOUSE.....	25
GENE MUTATION.....	26
CHROMOSOME EFFECTS	27
DNA DAMAGE	29
NEUROTOXICITY, HEN	29
NEUROTOXICITY, RAT	29
DEVELOPMENTAL NEUROTOXICITY, RATS.....	31
METABOLISM STUDIES	34
RISK ASSESSMENT/PROPOSITION 65 GENERAL SUBMISSIONS.....	36
OTHER.....	36

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT{tc \l1 "COMBINED, RAT}

****228-104 092157** Pettersen, J. C. and Richter, A.G. "Two-year chronic toxicity/oncogenicity study with R-4572 in rats" [Report No. T-13023]. CIBA-GEIGY Corp. Environmental Health Center, Farmington, Connecticut, 11/30/90. Fifty Crl: CD7 (SD) BR rats/sex were dosed for up to 2 yr with 0, 7, 40, or 300 ppm molinate (Lot No. EHC-0886-30/WRC-4921-8-22) in diet. Another group for 1-year interim sacrifice had 10/sex at 7, 40, and 300 ppm and 20/sex for controls and 600 ppm. Original review placed the NOAEL = 7 ppm, based on ovarian thecal/interstitial cell vacuolation and/or hypertrophy, which appeared slightly, but statistically significantly elevated at 40 ppm, and markedly increased in incidence and severity at 300 ppm. Later, a "blind" re-evaluation of ovarian slides (DPR Record No. 127478) by M. C. E. Hodge led to a higher NOEL of 40 ppm for ovarian thecal cell hypertrophy (see 1-liner below). Common findings at 300 ppm included: hindlimb ataxia and adduction, skeletal muscle atrophy (these signs were seen in both sexes but were more frequent in males); peripheral nerve (sciatic) degeneration in both sexes; distal spinal cord changes, especially eosinophilic bodies (presumed to be degenerating axons) in lumbar and sacral regions (both sexes) [eosinophilic bodies were also noted in brain (medulla) of several 600 ppm females]; modest cholinesterase (ChE) inhibition (of RBC ChE in both sexes, and of brain ChE in 300 to 600 ppm females: no associated clinical signs, even at 600 ppm); and modestly reduced hematocrits in both sexes. Oligospermia was noted in epididymides at 300 ppm, and testicular degeneration was noted at 600 ppm. There were adenomas or carcinomas in kidneys of five 300 ppm males vs. none in other groups: this was presumed to be treatment related. The neurological effects, the marked ovarian changes, and male kidney tumors are **"possible adverse effects"**. Decreased body weights and decreased food consumption were noted, along with increased survival, at 300 ppm in both sexes. This dose may therefore have exceeded the MTD. **Acceptable**, Aldous, 4/5/91, one-liner updated 6/21/94. (See follow-up studies below).

228-120 113132 (supplement to 228-104:092157). Horner, S.A., "Molinate: 10 day oral dosing study in rats", ICI Central Toxicology Laboratory, Cheshire, UK, Jan. 3, 1992. Five groups of 8 male Crl:CD(SD)BR rats were dosed by gavage for 10 days with either 4 ml/kg/day of corn oil vehicle, or with 15, 75, or 150 mg/kg/day molinate, or with 200 mg/kg/day 2,2,4-trimethylpentane (positive control). 150 mg/kg/day molinate led to some treatment-related deaths, associated with clinical signs such as subdued appearance, "hunched position", "sides pinched in", piloerection, ocular discharge, stains around nose and mouth, urinary incontinence, irregular breathing. Both 75 and 150 mg/kg/day molinate led to salivation. All 3 molinate groups had dose-related weight gain decrements. The 150 mg/kg/day group had reduced sperm in urine sediment (possibly treatment-related). Surviving 150 mg/kg/day rats had gross appearance of "reduced testis". All molinate groups had kidneys without visible hyaline droplets and without elevated a-2u-globulin upon microscopic examination. Assay for a-2u-globulin by immunoelectrophoresis found no significant increases in molinate groups, compared with marked elevation in positive controls. Thus the study does not indicate that male-rat-specific elevation of a-2u-globulin is related to the increase in kidney tumors noted in the referenced combined study. Aldous, 3/13/92.

071 085338, 072 090067, 086 088604, interim reports for 104:092157, above. No worksheet provided (G. Chernoff, 7/26/90).

228-144 127478 Hodge, M. C. E., "Two-year chronic toxicity/oncogenicity study with R-4572 in rats. Supplement to Stauffer Chemical Company Report Number T-13023. Histopathological re-evaluation of the ovarian thecal/interstitial cell vacuolation/hypertrophy", Zeneca Central Toxicology Laboratory, 9/22/93. This is a re-evaluation of ovarian slides of the two-year study, Report No. T-13023, DPR Record No. 092157. From the original report, it appeared that there might have been a treatment-related effect on incidence of ovarian thecal/interstitial cell vacuolation/hypertrophy as low as the 40 ppm level, therefore a re-examination was performed. The reviewing pathologist first re-examined all 600 ppm slides, finding that the original grading of ovarian changes was not applied uniformly. He made more specific sets of criteria to define three grades of ovarian thecal/interstitial cell vacuolation/hypertrophy. He then did a "blind" review of all available ovary slides (including 12-month interim sacrifice ovaries of all groups). The only incidences of ovarian thecal/interstitial cell vacuolation/hypertrophy discovered in this re-examination below 300 ppm were of "minimal" grade, and there were 6/69 controls, 3/59 in the 7 ppm group, and 10/60 in the 40 ppm group (see p. 22). There were clear responses in the 300 to 600 ppm groups, both in terms of incidence and degree. The pathologist made a valid conclusion that the NOEL for this effect was 40 ppm. Aldous, 6/21/94.

CHRONIC TOXICITY, RAT{tc \l1 "CHRONIC TOXICITY, RAT}

004 945346, "Ordram: Safety Evaluation by Repeated Oral Administration to Rats for 104 Weeks", (Woodard Research Corp., 6/3/77). Ordram technical (98.8%). Initial dosages of 0, 8, 16, and 32 mg/kg/day, reduced at week 18 to 0, 0.63, 2.0, and 6.32 mg/kg/day in diets of Fischer rats. Insufficient information to assess chronic or oncogenic potential. [Report cites dose-related increase in testicular weights in mid-dose and high-dose males, however this difference is apparently due primarily to differences in sizes of interstitial cell tumors in the last interim and term sacrifice groups. Interstitial cell tumors are very common in aged Fischer rats, and there is no evidence of compound effect on time to tumor in this study - C. Aldous, 3/5/87]. Study **Unacceptable**, not upgradeable: Far too little histology, too much loss of animals to autolysis, dose levels apparently far lower than justifiable. No further information required of this study. (J. Christopher, 3/5/85).

CHRONIC TOXICITY, DOG{tc \l1 "CHRONIC TOXICITY, DOG}

****228-097 095888** Pettersen, J. C. and Wadsworth, P. F., "One-year toxicity study with R-4572 in beagle dogs", Report No. T13236, CIBA-GEIGY CORP., Environmental Health Center, Farmington, CT, 12/17/90. Technical molinate, 97.6% purity, was administered in gelatin capsules daily at dosages of 0, 1, 10, 50, and 100 mg/kg/day. Duration of dosing was 1 year for all but the 100 mg/kg/day group, which was taken off treatment on day 106 and was administered empty capsules for the balance of the study. NOEL = 1 mg/kg/day (infrequent tremors and/or awkward gait in one or more dogs at 10 mg/kg/day, also very slight reduction in hematocrit in 10 mg/kg/day males at 3 and 6 months: see Section V. B. of review for details). Principal findings were neurological, and were the basis for identifying a "**possible adverse effect**". Common findings included clinical signs of ataxia, splayed

hindlimbs, reduced locomotor activity, tremor, abnormal voice, and noisy breathing in 50 and 100 mg/kg/day - recovery groups. Postural reactions, particularly of hindlimbs, were slightly depressed in these groups. These groups were generally hyperreflexic in patellar reflex tests. Many of the functional deficits observed in 100 mg/kg/day - recovery groups showed no signs of remission. Microscopic findings in brain included eosinophilic bodies or vacuolation, particularly in localized areas of medulla, in several 50 and 100 mg/kg/day - recovery dogs. Minimal to slight degrees of demyelination were observed in various levels of spinal cord, particularly at 50 mg/kg/day. There was slight demyelination of some peripheral nerves, particularly in 50 mg/kg/day dogs. Evidences of mild anemia included consistent reductions of RBC parameters (RBC count, Hb, HCT), noted in both sexes at 3 months in the 100 mg/kg/day - recovery dogs, decreased HCT values in 50 mg/kg/day males at 3 and 6 months, slightly increased RBC fragility in 50 mg/kg/day dogs, slight increases in reticulocyte counts in 50 mg/kg/day females, and modest evidences of RBC pigment accumulation in spleen and liver. Liver weights were elevated in 50 mg/kg/day dogs, and serum alkaline phosphatase and serum cholesterol were elevated in 50 mg/kg/day males, however liver microscopic findings were limited to slightly elevated Kupffer cell hemosiderin. **Acceptable**. Aldous, 4/18/91.

071 085340, 086 088606 interim reports for 097:095888, above. No worksheet provided (G. Chernoff, 7/26/90).

CHRONIC TOXICITY, MOUSE{tc \l1 "CHRONIC TOXICITY, MOUSE}

005 945347, "Ordram: Repeated Oral Administration to Mice for Lifetime", (Woodard Research Corp., 6/3/77). Ordram test article not characterized. Dosages of 0, 3.6, 7.2, and 14.4 mg/kg/day in diets of CAF1 mice. Insufficient data to qualify as a meaningful chronic study: "no toxicity of any kind observed", according to the reviewer [report indicates diminished survival in 14.4 mg/kg/day females near to scheduled term kill, however results not significant, nor were there other data to support treatment effects in any group]. Thus dose levels do not appear to be justified. Only 6 tissues routinely examined microscopically. Hematology/blood chemistry not done because of technical problems. No microscopic pathology of animals dying on study. **Unacceptable**, not upgradeable. No adverse effects indicated. (J. Christopher, 6/6/85).

005 945348 (Pathology report to 005 945347, above).

ONCOGENICITY, MOUSE{tc \l1 "ONCOGENICITY, MOUSE}

****228-107 096396** Potrepka, R.F., and Morrissey, R. L. "18-month dietary mouse oncogenicity study with R-4572" [Report No. T-13211]. CIBA-GEIGY Corp. Environmental Health Center, Farmington, CT, 1/14/91. Fifty CD-1 mice per sex per dose were administered technical molinate (97.6% purity, Lot No. EHC-0886-30/WRC-4921-8-22) in diet for 18 months. Doses were 0, 10, 100, 1000, and 2000 ppm. NOEL for males = 10 ppm (testicular degeneration). The NOEL for other effects was 100 ppm: this was based partially on neurological lesions [peripheral nerve demyelination and Schwann cell hyperplasia, and eosinophilic bodies (considered to be swollen, degenerated axons) in medulla and spinal cord]. Associated clinical effects such as hindlimb adduction, ataxia, hindlimb muscle

weakness, splayed hindlimb, and hindlimb atrophy were generally limited to 2000 ppm mice: females were particularly affected. Both sexes had adrenal degeneration at 1000 to 2000 ppm: this was most pronounced in females. Females had ovarian thecal/interstitial cell hyperplasia at 1000 and 2000 ppm: uterine atrophy, amyloidosis, or dilatation, as well as mammary gland atrophy were seen at 2000 ppm. Dose-related hematology changes, common at 12 and 18 months in both sexes, were decreases in RBC counts, Hb, and HCT. There was no treatment effect on neoplasia. The neurological effects and testicular lesions, and to a lesser extent, ovarian changes, are **possible adverse effects. Acceptable.** Aldous, 4/18/91.

071 085339, 088 088605 Interim reports for 096396, above.

REPRODUCTION, GENERAL COMMENTS{tc \11 "REPRODUCTION, GENERAL COMMENTS}

Many reproduction studies with molinate have been conducted in a total of 5 species. The most extensive data base is in rats, where a decrease in fertility, determined by decreased numbers of litters and implants, has been a consistent finding. EM studies of sperm preparations indicate that the primary lesion responsible for the adverse effect in male rats is associated with a disruption of the plasma membrane in the mid-piece region of maturing sperm. From this it has been reasoned that the decreased sperm count, motility, and viability observed in this species are associated with the mechanism which causes the membrane disruption.

In contrast to the findings in rodents, exposing rabbits to capsules of molinate had no adverse effect on fertility, or gross gonadal histology. In the monkey studies, only sperm parameters were measured. Using sperm collected by electroejaculation at weekly intervals over a 12 week period, no adverse effects from molinate exposure were seen on sperm count, motility, or percent normal. The individual variability of these parameters was extremely large from sample to sample in both monkey studies, and posed a major limitation in interpreting the results. This large variability can be attributed, in part, to the use of electroejaculation, a technique which can compromise sperm quantity, concentration, and quality.

The major source of human data were obtained as part of a large epidemiologic study on male workers at three molinate production plants. Fertility, calculated from retrospective male questionnaire data, and sperm parameters (count and percent normal) were unaffected by molinate exposure. The methods of questionnaire data collection, natality calculations, sperm collection, and sperm data analyses were severely flawed, thereby eliminating this study for use in a scientific risk assessment. [NOTE: These data were subsequently re-evaluated by Tomenson, J. and H. L. Northrop, Zeneca Report No. CTL/C/3097, DPR Document No. 228-174, Record No. 158168].

Many additional studies have been produced in recent years, providing valuable perspectives about mechanisms of rat reproductive toxicity and its relevance to other species, including man. A recent mechanistic study (DPR Record No.158171, Laboratory Study # CTL/R/1336, dated 11/05/97) provided evidence that the active metabolite is molinate sulfoxide. The primary mechanism involves inhibition of carboxylesterases which are essential for *in situ* production of testosterone in testicular interstitial cells, which make testosterone from circulating cholesterol esters derived from high density lipoproteins. Lack of testosterone evidently prevents normal sperm maturation, resulting in male

infertility. Another study (DPR Record No. 158170, Zeneca Report No. CTL/R/1343, 11/5/97) suggested that a similar process causes ovarian changes at low dose levels by a similar disturbance in steroidogenesis. Investigators state that man and most commonly used laboratory species do not rely on an analogous process for synthesis of reproductive hormones. Record No. 158171 (pp. 19-20) noted that humans also produce some molinate sulfoxide, however uptake of cholesterol in gonadal tissues in humans is not dependent on analogous esterases, but rather on acid hydrolysis of circulating low-density lipoproteins in lysosomes, hence comparable reproductive problems are not seen in humans. Thus, although epidemiological and reproductive effects studies in humans and non-rodent animals are not comparable to the rat studies in breadth and quality, none of the other species tested indicated remarkable reproductive toxicity. (Summary statements by G. Chernoff, 12/19/89 and 4/10/90; and subsequently updated by C. Aldous on 4/29/91 and 7/1/98 to reflect information in several recent studies).

REPRODUCTION, RAT{tc \l1 "REPRODUCTION, RAT}

There are many rat reproduction studies using molinate. The study by Moxon (1997) was designed to provide Noel's for known reproductive effects, based on experience derived from earlier studies. The resulting Noel's are 0.4 mg/kg/day for males (sperm abnormalities and reduced "day 1" live litter sizes at the LOEL of 0.8 mg/kg/day), and 1.9 mg/kg/day for females (based on ovarian vacuolation and hypertrophy at the LOEL of 4.7 mg/kg/day). The limitations of the use of rat reproductive toxicity as a surrogate for human risk characterization have been identified above. Aldous, 7/2/98.

Rat Multi-generation Studies: Both Sexes Treated{tc \l2 "Rat Multi-generation Studies: Both Sexes Treated}

****228-172 158166** Moxon, M. E., "Molinate: Two-generation reproduction study in the rat", Zeneca Central Toxicology Laboratory, 8/14/97, Laboratory Study # CTL/P/5409. Design included all elements of a standard reproduction study, with some modifications or additions. Purpose was to provide "NOEL's" for fertility reduction and other known reproductive effects. There were 40 pairs of Crl:CD (SD) BR rats/generation, with 1 litter for F0 parents, and F2a and F2b litters for F1 parents. Dietary dose levels of molinate (96.8%) were 0 ppm (both sexes); 5, 10, and 15 ppm (male: low, medium, and high); and 20, 50, and 300 ppm (female: low, medium, and high). All pairings were with parallel treatment groups (low with low, etc.). Respective dose levels (in mg/kg/day) were 0.4, 0.8, and 1.3 (F0 males); 0.5, 1.1, and 1.6 (F1 males); 1.9, 4.7, and 28.8 (F0 females); and 2.2, 5.6, and 34.5 (F1 females). Litters were not culled at day 4. Pups were examined daily for appearance of reproductive developmental landmarks (vaginal opening or preputial separation - all littering periods). Anogenital distances were measured in F2a pups on day 1 post-partum. Female adults were examined daily during the final 3 weeks of respective pre-mating periods by vaginal smear analysis in order to evaluate estrous cycling changes. At necropsy, epididymal sperm was examined for changes in motility and for altered morphology. Ovaries were examined by multiple sections to semi-quantitatively evaluate numbers and stages of development of oocytes. **Results:** There was no NOEL (for non-reproductive effects) in adult females [adrenal cortical vacuolation (F0) and reduced brain weights (F0 and F1) at the female LEL of 20 ppm]. Body weights were much reduced in females given 300 ppm molinate. The

non-reproductive NOEL for males was 10 ppm (reduced brain weight, F0 and F1). Reproductive NOEL for males = 5 ppm [sperm abnormalities, especially detached heads; and reduced live litter sizes on day 1 (gestation index) in all mating periods]. Reproductive NOEL for females was 20 ppm (ovarian interstitial tissue vacuolation and/or hypertrophy was dose-related in incidence and degree at 50 to 300 ppm). Fertility (live litters per mating) was reduced in high dose groups (statistically significant only for F2a littering period): this probably reflected primarily the 15 ppm treatment of males. **Acceptable, with possible adverse effects.** Aldous, 7/2/98.

003 945351, "Ordram Safety Evaluation by Repeated Oral Administration to Rats: Three Generation Reproduction Study", (Woodard Research Corporation, 6/3/77). Test article was Ordram, not further characterized. 0, 0.063, 0.2, and 0.63 mg/kg/day to males and females in 3-generation, 2 litter/generation study. Reduction in fertility at 0.63 mg/kg/day. Investigators concluded "survival of pups at the highest dose level may have been adversely affected" (not all reproduction performance data were provided., conclusion found on p. 5). Reviewer (Christopher) noted several serious deficiencies in this study. Not sufficient information to determine whether LEL was below 0.63 mg/kg/day. **Unacceptable**, not upgradeable. No further data needed from this study. (J. Christopher, 6/4/85).

003 945338. Pilot reproduction/fertility study. Possibly to set dose levels for study 003:945351 above. No review needed.

228-134 119319 (This is not a study, but is a discussion of appropriate NOEL's for reproductive effects). "Derivation of molinate NOEL's based on fertility effects", 11/30/92. Discussion states that ICI believes that the male fertility effects seen in rats are rodent-specific, and not relevant to humans, nevertheless bases for NOEL's for both sexes are presented. They estimated that 1 mg/m³ in inhalation studies was equivalent to 12 mg/kg/day absorbed dose, based on comparisons of urinary levels of the metabolite, 4-hydroxymolinate. They determined that toxicity was strictly a function of absorbed dose, whether by oral or inhalation route. Studies on male rat reproduction by either route were thus combined to determine the overall male rat reproductive effects NOEL. DPR review notes, however, that calculated equivalent doses by different routes did not yield comparable male reproductive toxicity. Investigators suggested that the male rat reproductive NOEL should be 3.6 mg/kg/day, based on their analysis of several LOEL's and NOEL's. This appears to be too high for a derived NOEL, since definitive male rat reproductive effects were observed at 4 mg/kg/day in study T-10421 (DPR Record No. 945355). In addition, Study KR1189 (DPR Record No. 127495) subsequently found a NOEL for male reproductive effects to be on the order of 0.5 mg/kg/day. Regarding female reproductive toxicity, the discussion considered the NOEL for ovarian thecal cell hypertrophy to be 50 ppm, but data suggest that 50 ppm is an LEL, and a conservative NOEL is 6 ppm (same as previous reviews). Aldous, 6/20/94.

228-144 127471 Wickramaratne, G. A., "Report No: CTL/L/5531. First revision to Molinate: The no-observed effect levels for male and female reproductive effects in rats; an overview", 11/18/93. This is a presentation of studies which have been previously reviewed. Items discussed included, among other things, (1) evidence that male reproductive effects resulted from failure of Sertoli cells to properly process sperm cells, (2) evidence that the ovarian thecal cell hypertrophy incidence increases found in the chronic study at doses up to 40 ppm were within historical control range. Investigators concluded

that the reproductive effects NOEL's should be at least 3.7 mg/kg/day for females and 1 mg/kg/day for males. There were no new data needing changes in DPR conclusions. Aldous, 6/21/94.

Rat Reproduction, Crossover Studies{tc \I2 "Rat Reproduction, Crossover Studies}

003 945353, "Ordram: Experiment to Show Whether the Male or the Female is Responsible for Reduced Fertility in Ordram Fed Rats", (Woodard Research Corp., 5/13/75). Ordram (not further characterized) at doses of 8, 16, or 32 mg/kg/day to either males or females. Only fertility studied. No litters sired by 16 or 32 mg/kg/day males, and only 2/8 litters in 8 mg/kg/day males. All treated females delivered litters. Infertility concluded to be a male treatment effect. **Unacceptable** to fill data requirement, but useful data. (J. Christopher, 6/4/85).

Rat Reproduction, Treatment Restricted to Females{tc \I2 "Rat Reproduction, Treatment Restricted to Females}

070 087658 "Two-Generation Reproduction Study in Female Rats with R-4572", (Ciba-Geigy Environmental Health Center, Report No. T-13218, 11/3/89). R-4572 Technical (Molinate), Lot #EHC-0866-30, 97.6%, was administered in the diet to groups of 25 female Sprague Dawley rats at dose levels of 0 (vehicle control of Purina Rat Chow and 0.1% corn oil), 6, 50, and 450 ppm for two generations. No clinical signs or necropsy findings suggestive of toxicity were observed at any of the dose levels tested and reduced food consumption at 450 ppm and weight gain at 50 and 450 ppm were attributed to poor palatability. Significant reductions in the fertility index and litter size were observed at 450 ppm, and vacuolation/hypertrophy of ovarian thecal/interstitial cells was reported at 50 and 450 ppm. A separate analysis of these data, provided in record no. 090134, reported a lack of association between the abnormal ovarian histopathology and a reproductive deficit, thereby necessitating the establishment of two separate NOEL's, one for systemic effects (abnormal ovarian histology), and one for functional effects (decreased fertility and reduced litter size). Reproductive systemic NOEL = 6 ppm (0.44 mg/kg) based on abnormal ovarian histology which is considered a **possible adverse effect**. Reproductive functional NOEL = 50 ppm (3.7 mg/kg/day) based on decreased fertility and reduced litter size. The study is acceptable as a supplemental study (G. Chernoff, 12/19/89; 1/24/90).

074 090134, "Evaluation of Two-Generation Study in Female Rats with R-4572", (G. A. Wickramaratne, ICI Americas Inc., Ref. T-13218, December 15, 1989). A supplemental report to 087658, consisting of a detailed re-appraisal of the histopathology data. The suggestion is made that since there is no association between the abnormal ovarian histopathology and the observed functional reproductive deficits, the NOEL for functional reproductive toxicity should be 50 ppm (G. Chernoff, 1/24/90).

228-138 121113 Nearly identical submission to 090134, above, with a few editing changes. Dr. Chernoff had already accepted the major argument of this evaluation, namely that there was no direct association between females with ovarian histopathological changes and females with fertility deficits (see 1-liner for Record No. 087658, above). No new DPR worksheet. Aldous, 6/16/94.

228-121 113585 Horner, J. M., "Molinate: Mechanistic study in the pregnant rat", Report No. CTL/T/2769, ICI Central Toxicology Laboratory, Cheshire, UK, March 3, 1992. Reproduction ancillary study, rat [relates to 228-003: 945351 and several ancillary studies]. Groups of 10 female Crl:CD(SD)BR rats were dosed with 0, 75, 135, or 200 mg/kg/day molinate (98.1%) by gavage (in 10 mg/kg corn oil vehicle) on days 7-9 of gestation. Investigators evaluated clinical observations, microscopic changes in ovaries and adrenals (with particular attention to changes in lipid content), and progesterone levels were measured at termination. High mortality in the higher two dosage groups limited the extent of parameters measured or reduced the precision of available data. Lipid content of adrenals was increased. Fatty cytoplasmic vacuolation was noted in adrenal zona fasciculata and zona reticularis, and in corpora lutea. No change in plasma progesterone was detected. The study did not confirm specific treatment responses which would account for female-mediated reproductive toxicity. Aldous, 5/1/92.

228-173 158167 Williams, J., "Molinate: An evaluation of vaginal opening in rat pups", Zeneca Central Toxicology Laboratory, Alderley Park, 8/8/97. Laboratory Report # CTL/P/5583. Groups of 40 Sprague-Dawley Crl:CD7(SD)BR females were dosed with 0 or 300 ppm molinate from day 7 *post coitum* to day 22 *post partum*. Pups were culled to 8/litter at day 8 and maintained on maternal diets throughout the study. Selected F1 pups were evaluated for growth, food consumption, and clinical observations for an additional 3 weeks, during which time the F1 females were examined daily for the time of vaginal opening. Groups of 40 selected F1 females were assigned to one of these four treatments: (1) 0 ppm molinate without estradiol treatment, (2) 300 ppm molinate without estradiol treatment, (3) 0 ppm molinate with estradiol treatment, (4) 300 ppm molinate with estradiol treatment. Estradiol treatment consisted of a single subcutaneous injection of estradiol benzoate (0.5 µg/rat) on day 28 *post partum*. All other F1 females received vehicle injections on the same day. F0 dams and F1 pups treated with molinate suffered modest body weight decrements during the pre-weaning period. Molinate-treated F1 females then continued to fall further behind controls in body weight, irrespective of estradiol treatment. Mean times for vaginal opening for the respective above treatment groups were 35.2, 39.5, 31.8, and 32.9 days. Vaginal opening times for groups 2-4 were all significantly different from untreated controls. Days of vaginal opening in both groups of estradiol-untreated rats corresponded closely to the achievement of about 100-110 g body weight in the majority of cases, such that the delays in vaginal opening in 300 ppm molinate rats corresponded to the delay in body weight gain at that dose level. Accelerated vaginal opening times in estradiol rats occurred prior to comparable body weight acquisition, however the 1-day delay in vaginal opening among molinate+estradiol rats compared to estradiol-only rats appeared to be a treatment response. There is no apparent reason to assign special significance to the delay in time of vaginal opening, which is consistent with a general delay in growth. Aldous, 7/2/98.

228-189 176745 Lovatt, C., "Molinate: effect on rat ovarian esterase activity," Central Toxicology Laboratory, Alderley Park, 7/14/00. Report No. CTL/00A120. Female SD rats (9-12 weeks), 3 per group, were dosed by gavage daily (4 mg/kg, corn oil vehicle) for 7 days at 0, 10, 40, 100, and 150 mg/kg/day, then killed after 24 hours. In addition, single-dose groups received either 0 or 40 mg/kg six hours before sacrifice. Ovaries were frozen at sacrifice, then homogenized to prepare a suspension in which esterase activity could be spectrophotometrically assessed using hydrolysis of *para*-nitrophenylacetate. All treatments caused inhibition of activity. Activities (as % of control) for

increasing dose levels in the repeat dosing study were 51, 38, 45, and 25 percent of control. Activity of the 40 mg/kg single dose group was 50% of control. Investigators determined that this inhibition of esterase activity may prevent reproductive tissues from acquiring cholesterol from high density lipoprotein in order to synthesize reproductive hormones (see discussion of Record No. 176746). Useful supplemental information. Aldous, 9/1/00.

Rat Reproduction, Treatment Restricted to Males (oral route only){tc \l2 "*Rat Reproduction, Treatment Restricted to Males (oral route only)*"}

228-177 158171 Wickramaratne, A., "Molinate: Elucidation of the processes underlying the reproduction effects in the male rat", Zeneca Central Toxicology Laboratory, Alderley Park, 11/05/97. Laboratory Study # CTL/R/1336. Investigators evaluated molinate and major metabolites with respect to several testicular functional and structural changes. Molinate and molinate sulfoxide elicited substantial but transient reductions in plasma and testicular testosterone levels *in vivo*. Other molinate metabolites, such as 4-hydroxymolinate, hexamethyleneimine, and molinate sulfone, did not alter plasma and testicular testosterone levels *in vivo*. Carboxylesterases found in the Leydig cells are necessary for these cells to take in cholesterol as a substrate for testosterone synthesis. Molinate markedly reduced such esterase activities in Leydig cells after *in vivo* administration. *In vitro* studies found that molinate sulfoxide was much more potent than molinate as a Leydig cell esterase inhibitor. Molinate sulfone was 2 orders of magnitude more potent than molinate sulfoxide as a Leydig cell esterase inhibitor *in vitro*, even though the sulfone had no *in vivo* effect on testosterone levels in plasma nor in interstitial fluid. Investigators proposed that the lack of *in vivo* effect of the sulfone may reflect rapid hydrolysis to hexamethyleneimine. ¹⁴C-molinate administered orally was found to concentrate in interstitial cells. When molinate (40 mg/kg/day) and molinate sulfoxide (20 mg/kg/day) were administered by implanted minipumps for 7 days, about 10% of epididymal sperm had mid-piece abnormalities. Hexamethyleneimine and 4-hydroxymolinate had no such effect. Investigators cited a parallel study which showed that molinate and molinate sulfoxide markedly reduced interstitial fluid levels of testicular precursors such as progesterone, 17 α -hydroxyprogesterone, and androstenedione, but 4-hydroxymolinate and hexamethyleneimine had no such effect. Data in this study are consistent with a mechanism of testicular toxicity in which molinate is first oxidized to molinate sulfoxide, which inhibits Leydig cell esterases necessary for uptake of cholesterol, a substrate needed for testosterone synthesis in interstitial cells. This paucity of testosterone disturbs the normal maturation of sperm, with mid-piece abnormalities as a major consequence. Useful supplemental information. All data were presented in summary format (usually figures). Aldous, 6/29/98.

228-183 164251 Ellis, M. K., A. G. Richardson, J. R. Foster, F. M. Smith, P. S. Widdowson, M. J. Farnworth, R. B. Moore, M. R. Pitts, and G. A. de S. Wickramaratne, "The reproductive toxicity of molinate and metabolites to the male rat: effects on testosterone and sperm morphology", *Toxicol. Appl. Pharmacol.* 151: 22-32 (1998). This is a summary of reproductive effects of molinate similar to Record No. 158171, above. Data were presented in summary format (usually figures), and are not amenable to independent review. Aldous, 11/17/98.

046 041544. Study T-10715, "A Comparison of the Effects of Benthicarb, Benthicarb Sulfoxide, and Ordram7 on Male Rat Fertility", (Stauffer, 4/14/82). Molinate, presumed technical, 0 and 20

mg/kg/day by gavage. No NOEL determined (mechanism study). Marked decrease in pregnancy index, and marked reduction in numbers of implants per pregnant dam. Sperm was damaged and reduced in number on maturation. Sperm cell membrane breaks were considered as possible cause of noted reproductive findings. **NOT APPLICABLE** to fill reproductive effects data gap, but useful data. Additional data requested. Confirms previously cited **ADVERSE EFFECTS** in male reproduction in rats. (C. Aldous, 7/28/86).

018 945355, "Ordram Fertility Study in Male Rats: Mechanism/Site of Action: T-10421", (Stauffer, 5/1/81). Molinate, technical (98.2%). Dosages varied between segments of study, but males were dosed with 0, 12, or 60 mg/kg/day for 5 days, or with 0, 0.2, 4, 12, or 30 mg/kg for 5 or 10 weeks. NOEL for 5-day treatment = 12 mg/kg/day (Reduction in fertility significant at week 3 post-treatment; also substantially reduced implants/pregnant female at week 3 and to a lesser extent at week 4). LEL for 10 week treatment with 0 or 12 mg/kg/day was 12 mg/kg/day (reduced fertility and reduced implantation). LEL for 5 week treatment with 0, 12, or 30 mg/kg/day was 12 mg/kg/day (reduced implantation). NOEL for 5-week treatment with 0, 0.2, and 4.0 mg/kg/day = 0.2 mg/kg/day (based on non-significant reduction in implantation; also decreased percentages of viable sperm, motile sperm; increased % abnormal sperm, decreased sperm cell count. All these findings down to 4 mg/kg/day). Serum hormone levels did not explain reproductive toxicity. **Unacceptable** as an independent study (not a guideline reproduction study), however useful data. No further data required for this study. (J. Christopher, 6/6/85).

228-144 127495 Hodge, M. C. E., "Molinate: Sperm morphology study in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 9/23/93. Study No. KR1189. Twelve CrI:CD(SD)BR males/group were dosed by gavage with 0, 0.5, 1, 2, 3, 4, or 8 mg/kg/day molinate (96.8%) daily for 35 days. Animals were killed, and sperm samples were taken from the right cauda epididymis for scanning EM evaluation. 100 sperm/rat were examined for various sperm abnormalities. Only incidence of midpiece abnormalities was remarkable: incidence with at least one mid-piece abnormality was 0, 1, 2, 6, 5, 10, and 12 for dose levels of 0, 0.5, 1, 2, 3, 4, or 8 mg/kg/day molinate. The higher 4 dose levels had multiple mid-piece abnormalities in 1, 3, 8, and 11 rats, respectively. Since there was one case of a mid-piece abnormality in the lowest dose group, but none in the concurrent controls, investigators re-examined unspecified numbers of sperm from all the rats in three studies, eventually finding one male having 3 sperm with similar lesions (Record No. 127496, this volume, Report No. CTL/L/5587 by F. M. Smith). Investigators considered 1 mg/kg/day to be the NOEL. This reviewer, however, has insufficient information to determine whether there is a NOEL at all, since existing data do not allow DPR to evaluate how unusual sperm mid-piece abnormalities are in control males. It is requested that registrants provide (1) the identities of the three molinate studies cited in CTL/L/5587 in which the sperm abnormalities have been examined by EM (the CTL Study Nos. do not appear to correlate with any recent male rat reproduction studies on file except for the present one), and (2) how many sperm/rat were examined in this search for abnormal sperm. Study is now **unacceptable**, but will provide useful information when requested data are received. Aldous, 6/21/94.

228-141 121889 Proposed protocol for study 228-144 127495, above.

003 945350, "Suppression of fertility in male rats: Ordram Technical", (Litton Bionetics, 10/29/76, LBI Project No. 2621). Ordram technical, lot RCK 0701. Feeding (or gavage during mating concurrent with treatment) of male rats with 0, 0.2, 1.0, or 5.0 mg/kg/day for two weeks. Matings on days 10-14 of treatment, also 2 and 4 weeks post-treatment. Apparent treatment effects at 5.0 mg/kg/day: decreased fertility, decreased # viable pups/litter, and slight but consistent increase in sperm agglutination. NOEL cannot be determined: numerous errors in study conduct, unusually high variability in control data. **Unacceptable** study, not very useful data, and superseded by other later studies. No more data required from this study. (J. Christopher, 6/4/85).

003 945352, "Ordram: Screening Study of Male Fertility and General Reproductive Performance in Rats Using Seven Thiocarbamate compounds", (Woodard Research Corp., 5/12/75). Ordram technical, lot RCK 0701. 0 or 32 mg/kg/day in diets of both males and females, 3 days prior to first mating of males, continuing treatment for 7 weeks, with second, third, and fourth matings in weeks 5, 6, and 7 of treatment. A final mating of males 5 weeks after termination of treatment. Results: Some litters sired in group mated on third day of treatment, however no litters sired with ongoing treatment during weeks 5-7. Apparent partial recovery at week 12 (5 weeks off treatment): 2 of 5 males sired litters. **Unacceptable** study with limited useful data and superseded by other later studies. No more data required from this study. (J. Christopher, 6/4/85).

003 945349. Summaries of studies to attempt to identify mechanism of testicular effects in rats. No definitive, documented studies found in this review. No worksheet generated.

228-181 162793 [Publication 1 of 2] Jewell, W. T., R. A. Hess, and M. G. Miller, "Testicular toxicity of molinate in the rat: Metabolic activation via sulfoxidation", *Toxicol. Appl. Pharmacol.* **149**, 159-166 (1998). Single ip injections of molinate to male Sprague-Dawley rats caused testicular toxicity (200 or 400 mg/kg was measurably toxic: 100 mg/kg was not). The high dose caused the most rapid response (2 days, vs. 7 days at 200 mg/kg). Initial changes included abnormally shaped spermatid heads, spermatocyte nuclear fragmentation, degeneration of spermatocytes and spermatogonia, and failed spermiation. After a week, there was severe disorganization of seminiferous epithelium, with loss of germ cells and abnormal appearance of Sertoli cells. The molinate sulfoxide metabolite elicited damage at 200 mg/kg similar to that of molinate at 400 mg/kg. There was appreciable formation of molinate sulfoxide from molinate in microsomes derived from liver and testes. The sulfoxide was also relatively abundant in blood after molinate administration. The molinate sulfone metabolite was not detected in tissues. Although administration of the sulfone caused severe testicular toxicity, the pattern of pathology was greatly different from that of molinate or the sulfoxide. The article provides useful summary data and figures, but does not address data requirements and is not sufficiently detailed in presentation to be "reviewable". Aldous, 11/18/98.

228-181 162793 [Publication 2 of 2] Jewell, W. T. and M. G. Miller, "Identification of a carboxylesterase as the major protein bound by molinate", *Toxicol. Appl. Pharmacol.* **149**, 226-234 (1998). ¹⁴C-labeled molinate, its sulfoxide, and its sulfone metabolites were incubated with microsomal preparations made from liver or testes of Sprague-Dawley rats. Proteins were purified, then chromatographed by SDS-PAGE. This technique revealed only one strong band, found at ca. 60,000 Daltons, from both tissues. Native gel analyses showed that this protein is a trimer, hence about 3 times

that size in native form. Phenylmethylsulfonyl fluoride (PMSF) blocked binding of molinate and of both metabolites to this protein. PMSF is known to bind tightly to serine esterases, and results suggested that molinate and its metabolites do likewise. Further analyses (refined MW estimation, isoelectric focusing, sequence analyses of the first 17 N-terminal amino acids) suggested that the bound protein was Hydrolase A, a carboxylesterase previously identified in liver and testes. Investigators assayed "nonspecific esterase" (NSE) activity in microsomal preparations of liver and testes. The sulfoxide was the most potent inhibitor, with an IC value of 2×10^{-6} M in both preparations, compared to 10×10^{-6} M and 40×10^{-6} M for the sulfone in preparations of testes and liver, respectively, and 200×10^{-6} M and 150×10^{-6} M for molinate in respective preparations. Testis esterase activity was evaluated 1 hr after dosing with 400 mg/kg/day molinate ip. Frozen sections were treated by a commercial esterase activity staining procedure. NSE staining was strong in testicular interstitium (where Leydig cells are found) in control sections, but absent in sections from molinate-treated rats. Hydrolase A had been shown to constitute about 10% of cellular protein in Leydig cells. Investigators concluded that the inhibition of this enzyme by molinate and most particularly by its sulfoxide was the basis of molinate toxicity, perhaps by inhibiting mobilization of cholesterol esters, leading to reduction of testosterone synthesis. The article provides useful summary data and figures, but does not address statutory data requirements and is not sufficiently detailed in presentation to warrant a DPR worksheet. Aldous, 11/19/98.

228-189 176746 Lovatt, C., "Molinate: effect on rat testicular esterase activity and testosterone levels" Central Toxicology Laboratory, Alderley Park, 7/20/00. Report No. CTL/00A121. Male SD rats, 6/group, were dosed once with 0, 6, 12, or 25 mg/kg molinate (96.9%) by gavage (4 mg/kg, corn oil vehicle) six hours before sacrifice. Blood and testicular interstitial fluid were assayed for testosterone using radioimmune assays. Testes were frozen upon collection, then homogenized in a buffer to prepare a suspension in which esterase activity could be assessed spectrophotometrically using hydrolysis of *para*-nitrophenylacetate. All three parameters were affected over the entire range tested. The following outcomes were obtained as percent of control for low to high dose levels, respectively: (plasma testosterone) 34, 10, and 9%; (testicular interstitial fluid) 69, 34, and 21%; and (testicular esterase activity) 10, 9, and 4%. Investigators determined that this inhibition of esterase activity may prevent reproductive tissues from acquiring cholesterol from high density lipoprotein in order to synthesize reproductive hormones. The association between levels which inhibited esterase activity and levels which reduced circulating and interstitial fluid testosterone levels was an evidence of the functional relationship between these effects. Useful supplemental information. Aldous, 9/1/00.

Rat Reproduction, Treatment Restricted to Males (inhalation route){tc \l2 "Rat Reproduction, Treatment Restricted to Males (inhalation route)}

The following studies are of questionable value in risk assessment, since the average absorbed values are grossly in excess of theoretical values based on inhalation alone. See especially Document No. 228-134, Record No. 119319. Aldous, 7/2/98.

046 041546 Study T-10189, "Evaluation of Male Fertility Following Inhalation Exposure to Ordram7 Technical in Rats", (Stauffer, 8/13/82). Molinate, technical 0, 0.1, 0.6, 1.8, and 4.0 mg/m³, 6 hr/day, 5 days/wk, 13 week exposure by inhalation. No NOEL was observed for reproductive nor for

non-reproductive effects [necrosis in spermatids and/or spermatocytes (same grade of response in all treated groups), also necrotizing rhinitis (grade of response increased with dose); both at all dosages after termination of treatment. Decreased pregnancy index in 0.6 mg/m³ group and above, and decreased implantations at 1.8 mg/m³ and above at termination of treatment. Full recovery from rhinitis and partial reversibility of reproductive effects in 2-month recovery animals.) **Unacceptable** and not upgradeable to fill data requirements, but useful data. (C. Aldous, 7/30/86).

046 041547 Study T-10494, "Evaluation of Male Fertility Following Four-Week Inhalation Exposure to Ordram Technical in Rats", (Stauffer, 1982). Molinate, technical 0, 0.1, 0.2, 0.3, 0.6, and 1.6, mg/m³, 6 hr/day, 5 days/wk, 4 week exposure by inhalation. Apparent NOEL = 0.3 mg/m³ (higher percentage of "abnormal sperm" and reduced percentage of motile sperm at 0.6 and 1.6 mg/m³. Reduced numbers of implants at same levels.) **Unacceptable** and not upgradeable to fill data requirements, but useful data. (C. Aldous, July 30, 1988).

003 028492, "A 13 Week Inhalation Toxicity Study and Reproduction-Fertility Study of R-4572 in the Rat", (Biodynamics, 12/12/79, Project Nos. 78-7153 & 78-2346, also designated T-10003). Inhalation exposure (respirable size) to 0, 2.2, 11.1, or 42 mg/m³, 6 hr/day, 5 days/wk. No NOEL observed (decreased sperm count and abnormal sperm, also decreased implantation rate at LDT of 2.2 mg/m³). **Unacceptable** study to fill data requirements, but useful data. (J. Christopher, 6/4/85).

Rat Reproduction, Related Studies, Acute to Subchronic {12 "***Rat Reproduction, Related Studies, Acute to Subchronic***}

228-176 158170 Wickramaratne, A., "Report No. CTL/R/1343 - The morphological effects of the thiocarbamate herbicide, molinate, on the ovary, adrenal and testis of the Sprague-Dawley rat", 11/5/97. This is a synopsis of a study, for which there does not appear to be a full report available at this time. This record has a brief text section, one table (male organ weights), and a few photocopies of light micrographs. In lieu of a worksheet of this preliminary submission, the following information is quoted from the abstract. "Female rats were dosed by gavage with molinate at 0, 10, 40, 100, and 150 mg/kg/day for 7 days while male rats were dosed with molinate at 0, 10, 30, and 60 mg/kg/day for 35 days and the histological effects on the target organs and sperm of the male rat observed. Following treatment with molinate at 100 and 150 mg/kg/day for 7 days, adrenal cortical and ovarian interstitial cells in the female rats were found to accumulate lipid and become hypertrophied. These effects were not seen at 40 mg/kg/day and below. In the male rats testicular atrophy and necrosis of the spermatid cell population, primarily in stage VIII tubules, was observed at 30 and 60 mg/kg/day and sperm, taken from the epididymis, showed a distinct abnormality of the head-midpiece junction. A no-effect level for these changes was seen at 10 mg/kg/day. The target specificity and character of the lesion produced by molinate in the ovarian interstitial cells and adrenal cortex suggested a block in steroidogenesis and the specificity of the effect to the stage VIII tubules of the testes, a stage highly dependent upon adequate concentrations of testosterone to achieve separation of sperm from Sertoli cells, support the hypothesis that the changes seen were mediated via an abnormality in steroidogenesis." Aldous, 6/30/98.

228-185 168300 Ellis, M. K. and M. J. Farnworth, "Molinate: effect of molinate and molinate

metabolites on plasma and testicular interstitial fluid hormone concentrations in the rat *in vivo*", Zeneca Central Toxicology Laboratory, Cheshire. Cover letter was dated 4/13/99. Male CD rats were dosed with varying amounts of 5 compounds: (1) molinate, (2) molinate sulfoxide, (3) molinate sulfone, (4) 4-hydroxymolinate, and (5) hexamethyleneimine. Sacrifice time after dosing was varied, however maximal responses to the first two chemicals above occurred at about 6 hr, hence responses at this time interval proved most important for comparisons. Molinate at 40 mg/kg and 20 mg/kg molinate sulfoxide caused large reductions in plasma levels of androstenedione and testosterone, particularly at 6 hr post-dosing. There were no consistent plasma hormone changes for the other three test chemicals. Measurements of interstitial fluid levels of hormones and intermediates revealed a broader array of responses to both molinate and molinate sulfoxide: both agents caused comparable reductions of testosterone, progesterone, 17a-hydroxyprogesterone, and androstenedione. Degrees of response were similar when comparing 40 mg/kg molinate and 20 mg/kg molinate sulfoxide. There were no consistent responses to molinate sulfone or to 4-hydroxymolinate. At a dose level of hexamethyleneimine stated to be highly toxic (40 mg/kg), there were reductions in levels of testosterone and progesterone, but not of intermediates between them. Data suggest that hexamethyleneimine is not an important contributor to toxicity associated with perturbations in testosterone synthesis, and that molinate sulfoxide may be the primary or exclusive ultimate toxic species. Aldous, 12/29/99.

228-186 170474 Re-submission of 228-185 168300, with individual data for rat hormone levels. No new DPR worksheet. Aldous, 12/9/99.

228-185 168301 Ellis, M. K. and M. J. Farnworth, "Molinate: investigation into the mode of action in the rat Leydig cell *in vitro*", Central Toxicology Laboratory, Cheshire. Final Report Date was not given, cover letter date was 4/13/99. Report No. CTL/R/8609. Leydig cells obtained from CD rats were used *in vitro* to assess how molinate inhibited testosterone synthesis. Cells were suspended to about 10^5 cells/ml, and placed in 24-well tissue culture dishes in "Minimum Essential Medium" containing BSA. Effects of molinate and molinate sulfoxide on testosterone synthesis were evaluated in the presence of varying amounts of testosterone precursors. A known inhibitor of testosterone synthesis, ketoconazole, was the positive control. Investigators also evaluated inhibitory effects of molinate, molinate sulfoxide, and molinate sulfone on cholesterol ester hydrolase activity. **Results:** cholesterol addition to cell suspensions did not increase testosterone synthesis, however added pregnenolone, progesterone, 17a-hydroxyprogesterone, and androstenedione increased testosterone synthesis in positive and negative control suspensions, and in the presence of 400 μ M molinate or molinate sulfoxide. Addition of 22-hydroxycholesterol gave smaller increases in testosterone synthesis, possibly due to poor uptake into cells. Results indicate that molinate inhibits testosterone synthesis at a step before pregnenolone formation. Molinate was comparatively insensitive on inhibiting cholesterol ester hydrolase activity in Leydig cells, with less than 50% inhibition at 400 μ M. By comparison, molinate sulfoxide and molinate sulfone achieved 50% inhibition at approximately 250 nM and 2.5 nM, respectively. Observations are consistent with molinate sulfoxide being the primary inhibitor of testosterone synthesis by inhibiting cholesterol ester hydrolase activity. Supplemental data. Aldous, 12/29/99.

228-186 170475 Re-submission of 228-185 168301, with individual data each *in vitro* analysis. No new DPR worksheet. Aldous, 12/9/99.

228-185 168302 Ellis, M. K. and M. J. Farnworth, "Molinate: effect of molinate and molinate metabolites following seven day administration on testis and sperm morphology in the rat", Central Toxicology Laboratory, Cheshire. Final report date was not given: cover letter date was 4/13/99. Report # CTL/R/8608. Male CD rats, 4 rats/group, were implanted for 7 days with osmotic mini-pumps set to deliver (1) PEG200 (vehicle control), (2) molinate treatments (40 and 140 mg/kg/day), (3) molinate sulfoxide treatments (10 and 20 mg/kg/day), (4) 4-hydroxymolinate (10 mg/kg/day), or (5) hexamethyleneimine (10 mg/kg/day). Epididymal sperm samples were processed for light microscopy in all groups, and for scanning EM microscopy in all but the 40 mg/kg/day molinate and the 10 mg/kg/day molinate sulfoxide groups. Both light microscopy and EM evaluation found sperm mid-piece abnormalities in all molinate and molinate sulfoxide groups (6% to 12% in the range evaluated, suggesting that molinate sulfoxide may be slightly more potent than molinate in this respect). EM studies characterized the mid-piece lesion as a disruption of the cell membrane in the region where the head is attached. In some micrographs, microfilaments could be seen protruding from the disrupted membrane. Mid-piece abnormalities were absent from all other groups except for a very low incidence in the 4-hydroxymolinate group (about 15-fold lower incidence than the lowest incidence in any molinate or molinate sulfoxide groups). Since mid-piece abnormalities appear to be quite uncommon in control rats, this suggests a weak response of the same type observed after molinate treatment. Incidences of mid-piece abnormalities in molinate and molinate sulfoxide groups were about 20-fold higher in the light microscopy evaluations compared to EM. The report did not discuss reasons for such a difference. Further, the light microscopy evaluations found substantial increases of detached heads in all molinate and molinate sulfoxide groups, and an appreciable increase in tail abnormalities in the 140 mg/kg/day molinate group. Supplemental data. Aldous, 12/2/99.

228-186 170476 Re-submission of 228-185 168302, with the addition of individual data for sperm abnormality counts by light microscopy and EM, also individual testes weights. No new DPR worksheet. Aldous, 12/9/99.

228-185 168303 Foster, J. R., "Neutral cholesterol ester hydrolase: a key enzyme in the control of steroidogenesis in rodents". This is a review article describing the importance of this enzyme in rodents, particularly relating to utilization of cholesteryl esters from circulating HDL, the primary source of cholesterol for steroidogenesis in rodent Leydig cells. In man, the primary source of cholesterol for steroidogenesis is LDL, and release of cholesterol occurs by lysosomal acidic hydrolases. Thus the strong inhibition of neutral cholesterol ester hydrolase in rats by molinate and molinate sulfoxide (see Record No. 168301) would not be expected to be an issue for human reproductive toxicity. No new data, hence no DPR review. Aldous, 12/2/99.

REPRODUCTION, MOUSE{tc \l1 "REPRODUCTION, MOUSE}

018 945354, "Ordram Antifertility Study in Mice: T-10121", (Stauffer, 12/80). Molinate, technical (98.2%). 7-week gavage treatment of male CD-1 mice with 0, 2, 20, 100, or 200 mg/kg/day. NOEL = 20 mg/kg/day (decreased implantations per pregnant female and decreased fertility at 100

mg/kg/day). **NOT ACCEPTABLE** as an independent study (not a guideline reproduction study), however useful data. No further data required from this study. (J. Christopher, 6/6/85).

REPRODUCTION, MONKEY

228-098 095839 Zühlke, U., and Bee, W.; "Molinate: Evaluation of sperm morphology in the Cynomolgus monkey". Hazleton Laboratories Deutschland GmbH, Jan. 7, 1991. Ten monkeys per group were dosed with 0, 0.2, 10, or 50 mg/kg/day of molinate (Batch BJB 2605) by gavage (corn oil vehicle) for 12 weeks. There were no changes in sperm morphology identified, nor were any "adverse effects" indicated. The study is of very limited value for evaluating possible effects on sperm morphology, due to small numbers of animals and great variability in measured parameters. There is no apparent reason to submit additional information regarding this study. Aldous, 4/22/91. NOTE: A new submission was received in May, 1991: Document No. 228-111, Record No. 097046. This is presumably the information requested and examined by Dr. Chernoff, who had left this Branch prior to receipt of the photographs, and who is currently not available for comment. He did not prepare a written review for CDFA/DPR Branch records. Aldous, 5/4/92.

228-105 096314 Statement by Dr. Themann, indicating that notches in sperm head-neck area are normal in Cynomolgus monkeys and in man, followed by title page to text: Ultrastructure of Human Gametogenesis and Early Embryogenesis, Blerkom, J. V. and Motta, P.M., Eds., Kluwer Academic Publishers, Boston, 1989. A photocopy of two human sperm is included, submitted to address the issue of notches; nevertheless the captions do not mention notches, nor could any be seen in this reproduction. C. Aldous, 4/19/91 (no worksheet).

228-110 096794 A single page of EM summary tables of sperm morphology, qualitatively similar to p. 119 of study 095839, above, from which study these data came. Electron micrographs corresponding to these tables have been submitted to Dr. G. Chernoff of Calif. DHS. No additional review is relevant at this time. Aldous, 4/22/91.

228-092 088921 Interim report for 098:095839, above (no worksheet).

228-105 096315 The only data in this 2-page submission are historical data for Cynomolgus monkeys: sperm count and ejaculate weights. C. Aldous, 4/19/91 (no worksheet).

228-105 096316 SOP for "Ejaculate collection and evaluation in primates", with light microscopic analysis. C. Aldous, 4/19/91 (no worksheet).

228-105 096319 SOP for "Preparation of spermatozoa for scanning electron microscopy". C. Aldous, 4/19/91 (no worksheet).

228-105 096320 SOP for "Investigation of spermatozoa morphology by light microscopy". Criteria for counting abnormal spermatozoa. C. Aldous, 4/19/91 (no worksheet).

228-105 096321 Copy of part of chapter 2, "Collection and examination of human semen", from WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction, Cambridge University Press, New York, 1987. Included are rough photocopies of several plates showing various sperm abnormalities. C. Aldous, 4/19/91 (no worksheet).

019 945356, "The Effect of Ordram7 on Nonhuman Primate Sperm Production", (Stauffer, 12/16/81). T-10714. Ordram, technical (98.6%), 0, 0.2, 10.0, and 50.0 mg/kg/day, 5 days/week, for 12 weeks. Administration by oral gavage (with nasal gastric tube) in corn oil vehicle to male monkeys (Macaca fascicularis). No evidence of untoward reproductive effects, based on sperm analyses and hormonal levels. High dose sufficient to substantially reduce plasma cholinesterase levels. **NOT ACCEPTABLE** in lieu of a full reproduction study, however useful data. (J. Christopher, 6/4/85).

REPRODUCTION, RABBIT{tc \l1 "REPRODUCTION, RABBIT}

228-128 118022 Tinston, D. J., "Molinate: Fertility study in male rabbits". Report No. CTL/P/3225. This was the first of three efforts to conduct a full-scale evaluation of fertility effects of molinate in male rabbits. This study was terminated prematurely due to high losses of rabbits in the two higher dose groups. Study began with 10 NZW males/group dosed by gavage with 0, 10, 100, or 200 mg/kg/day. The 200 mg/kg/day group was terminated before the first semen evaluation and fertility trial, which was scheduled at week 4. Three were found dead by day 12 and 3 more were killed in extremis by day 13. The 100 mg/kg/day group was maintained through the week 4 trial, however 4 of that group died or were killed in extremis by that time. Females inseminated by the 100 mg/kg/day group survivors had a statistically significantly elevated pre-implantation loss compared to controls (46% vs. 19%). Incidence of sperm mid-piece abnormalities was also elevated in that group. The latter finding was based on light microscopy, whereas EM did not confirm any increase in abnormal sperm. This study gave an apparent NOEL of 10 mg/kg/day, however later studies (particularly the third of this series: DPR Record No. 118042, ICI Report No. CTL/P/3684) were more successful in assessing male rabbit reproductive effects. Study is **not acceptable**, with limited useful data. Since fertility and apparent sperm morphology change occurred only at a relatively high and systemically toxic dose, no "adverse effects" are indicated. Aldous, 6/15/94.

228-108 092643 (ancillary rabbit reproduction) "Molinate: Second fertility study in male rabbits". CTL Study No: RB0533. ICI Central Toxicology Laboratory (Cheshire, UK). The record in Vol. 108 was the March 18, 1991 interim summary report (not QA - approved). The complete, QA-approved report is in 228-113 089637 (dated 6/6/91). There were no essential changes in the final report from the preliminary report, which was initially reviewed by Medical Toxicology. Ten NZW male rabbits/dose were administered 0, 10, 100, or 200 mg/kg/day daily by gavage. Males were mated 1/1 with untreated does on weeks -1 (pretreatment), 4, 8, and 12. Does were C-sectioned on gestation day 18 to evaluate fertility. At comparable intervals, semen samples from the males were collected for sperm numbers, morphology, motility, and scanning electron microscopy. Males were killed at week 13, and epididymal semen was collected for similar analyses. Blood was sampled at weeks 4, 8, and 12 for RBC cholinesterase (ChE) inhibition, and brains were tested for brain ChE at termination of males. There were no apparent clinical abnormalities. Body weights of 200 mg/kg/day

males trailed slightly behind other groups (not definitive evidence of a treatment effect). There was no apparent ChE effect. There were a few mortalities: 3 of the 200 mg/kg/day males and 1 of the 100 mg/kg/day males were presumed by investigators to have died due to molinate. Deaths of 5/10 200 mg/kg/day males by week 12 limited the statistical power of this study, however mean numbers of live fetuses in the 200 mg/kg/day group at week 12 were significantly lower than controls. Apparent (non-significant) increases in pre-implantation and post-implantation losses seemed to account for the reduced numbers of live fetuses at that dose. Plasma membrane abnormalities of the mid-piece region of epididymal sperm were quantified, and there were no significant differences, although mean numbers of mid-piece abnormalities were somewhat elevated in the 200 mg/kg/day group. Study is inconclusive: neither proving nor disproving putative male fertility (including sperm morphology) effects in rabbits. No new "possible adverse effect" is indicated. **Not acceptable** (ancillary study by design: the data gap for reproduction study is already filled). A repeat study is being planned, and a protocol summary (228-115 092997) was submitted on 7/22/91 for Medical Toxicology Branch comment. Aldous, 4/19/91, 8/01/91. (See also 116:098509, below).

228-116 098509, 098510 (photographic supplements to ancillary rabbit reproduction to study 108:092643), above. Supplement provides representative light micrographs and scanning electron micrographs of normal and abnormal sperm, as examined in the ancillary study. This makes the report "complete" (no more data are requested relating to record 092643). The study is not upgradeable, nor is it required to fill a data gap. An elective replacement study in this species is currently being proposed. Aldous, 10/15/91.

228-106 096313 less complete report in comparison to 092643, above. No review is needed for this report. Aldous, 4/9/91.

228-100 092018 Interim report for 092643, above. No review is needed for this report. Aldous, 4/22/91.

085 086900, Protocol for study 108:092643, above.

228-113 089637 This is the complete report for the "Second Fertility Study" in rabbits, which was reviewed as an interim report in 228-108:092643 (see above). There were no substantive changes in this report over what has been previously reviewed. Investigators continue to determine that study is inconclusive, and recommend a replacement study. No new worksheet is needed. Aldous, 7/24/91.

228-092 088922 Rose, P.H., "Molinate: Overview of three studies in male rabbits conducted at CTL". Three studies, taken together, appear to support the choice of dose levels used in study 228-108:092643, above. Four of 5 male NZW rabbits administered 300 mg/kg/day molinate in corn oil died or were killed in extremis after extensive body weight losses were noted during a 28-day study. A subsequent 28-day study found no mortalities and no clinical signs of toxicity up to the highest dose tested of 250 mg/kg/day. A sharp dose-response curve was inferred, and 200 mg/kg/day was considered to be the highest dose likely to be sustainable for a 12-week study. Aldous, 4/22/91 (no worksheet).

228-113 089612 Tinston, D. J. "Molinate: Preliminary study in male rabbits". ICI Central Toxicology Laboratory, 5/31/91. This is one of the 3 studies discussed 092:088922, above. Five male NZW rabbits/group were dosed with 0, 100, 200, or 300 mg/kg/day molinate for 28 days. High dose was not tolerated: 4/5 of 300 mg/kg/day males died or were killed in extremis. Doses up to 200 mg/kg/day seemed tolerable. No worksheet. Aldous, 7/24/91.

228-113 089636 Tinston, D. J. "Molinate: Preliminary study in male rabbits". ICI Central Toxicology Laboratory, 5/31/91. This is one of the 3 studies discussed 092:088922, above. Similar in design to study 089612: this study employed dose levels of 0, 40, 100, and 250 mg/kg/day. The only mortality was one 100 mg/kg/day rabbit. There were no indications that these doses exceeded tolerated range. No worksheet. Aldous, 7/24/91.

228-130 118042 Tinston, D. J., "Molinate: Third fertility study in male rabbits", ICI Central Toxicology Laboratory, Oct. 1, 1992. Report No. CTL/P/3684. Males were dosed by gavage with 0, 40, 80, or 160 mg/kg/day molinate. (The high dose was reduced to 120 mg/kg/day after week 5 due to unacceptable mortality). Study endpoints included fertility and examination of sperm morphology. Males were evaluated pre-dose (week -7), and at weeks 5, 9, and 13. Evaluations included sperm number and motility (light microscopy), sperm morphology of fixed samples (light microscopy), sperm morphology of fixed samples (electron microscopy), and fertility after insemination of untreated females. There was no systemic toxicity NOEL for treated males. Apparently deaths due to treatment were dose-related at all dose levels. Also, RBC cholinesterase was inhibited slightly but statistically significantly at all dose levels. There was a NOEL of 40 mg/kg/day for sperm morphology, based on atypical staining of sperm heads under light microscopy (no corresponding changes under EM). No NOEL could be established for transient pre-implantation loss (evident without dose-response relationship in all treatment groups at weeks 5 and 9, but not at week 13). There was a serious confounding effect due to the choice of gavage dosing, leading to a substantial period of inappetence irrespective of treatment. Thus, a largely inconclusive study provides some useful information, since data suggest that rabbits do not suffer sperm mid-piece abnormalities comparable to rats. Data quality is insufficient to establish an "adverse effect". Aldous, 6/30/94.

228-135 119909 Addendum to Record No. 118042, above (providing standard deviations for litter data). New statistical information does not impact study interpretation. No DPR worksheet. Aldous, 6/15/94.

228-123 113612 Interim report to Record No. 118042. No worksheet needed.

228-124 115786 Interim report to Record No. 118042. No worksheet needed.

228-131 118004 Wickramaratne, G. A., "Molinate: Overview of fertility studies in male rabbits", Oct. 1, 1992. A discussion of major findings of the four principal rabbit reproduction studies: DPR Record Nos. 945357, 118022, 092643, and 118042. Inconsistencies in degrees of toxicity to treated males between studies, as well as differences in the types of sperm abnormalities (including lack of agreement between light microscopic evaluation and EM evaluation) or differences in reproductive outcomes (such as pre-implantation losses) were offered as evidence that "A rigorous and unique investigation into the

potential effects on reproduction in rabbits of molinate has failed to demonstrate any consistent adverse effect". Not a study, nor was new information presented, hence no DPR review. Aldous, 6/15/94.

077 (no record number), "Molinate-Further Investigations of Male Fertility in Rabbits and Non-human Primates. Rationale for ICI Modifications of CDFA-Proposed Designs", (ICI Central Toxicology Laboratory, 2/7/90). This brief report presents the rationale for modifying the designs of rabbit and non-human primate studies, proposed by CDFA to further test the hypothesis that the adverse reproductive effect induced by molinate is a rodent specific phenomenon. CDFA agrees with the rationale and accepts the modifications with one exception. The analysis of epididymal sperm (motility, viability, count, and morphology by light microscopy and SEM) is still considered desirable, since it will allow for a comparison with similar data obtained in previous rat studies (G. Chernoff, 3/23/90).

228-022 945357, "Ordran Antifertility Study in Rabbits", (Stauffer, 11/80). [T-10176]. Ordran technical (98.2%). 0, 2, 20, and 200 mg/ml daily to males only for 6 weeks in gelatin capsules. Corn oil vehicle used except in undiluted high dose group. "NOEL" = 200 mg/kg/day (HDT). No effects on parameters studied: mating and fertility indices, litter size, pup weight, gestation length, pup viability. **NOT ACCEPTABLE** (not a complete reproductive study), but useful data. No more data required from this study. (J. Christopher, 6/4/85).

082 090585, "Molinate: Preliminary Study in Male Rabbits", (ICI Central Toxicology Laboratory, Study Number RB0509, 4/17/90). A brief summary of the pilot study used to justify the doses for rabbit study 108:092643. No worksheet provided (G. Chernoff, 5/9/90).

REPRODUCTION, HUMAN EPIDEMIOLOGICAL STUDIES{tc \l1 "REPRODUCTION, HUMAN EPIDEMIOLOGICAL STUDIES}

228-001 019849-019852, "Epidemiologic Assessment of Fertility in Male Workers Exposed to Ordran at the Stauffer Chemical Co.", (University of Rochester, April 20, 1984). Male workers from three plants were evaluated for adverse reproductive effects from working with Ordran. Retrospective fertility data collected from the men were used to calculate natality (observed/expected birth rate), which did not differ between the various exposure groups. Exposure dosage was estimated by multiplying the number of hours exposure by monitoring data on breathing zone concentrations. Monetary incentives were used in soliciting sperm samples which were analyzed for sperm count and percent normal sperm as measured by light microscopy. Comparison of groups by change in exposure hours with change in sperm count or percent normal sperm gave equivocal results. Multivariate regression analyses erased any association between Ordran exposure and altered sperm parameters. The power of this study, along with the validity of the conclusions, is severely limited by the procedure used to collect the reproductive histories, the methodology used for determining natality, the methodology used for estimating exposure levels, the validity of the sperm count data, the statistical procedures for analyzing sperm count and percent abnormal sperm, the lack of data on sperm motility, and the absence of electron microscopic evaluation of sperm morphology. Because of these limitations, the study is considered inadequate for risk assessment purposes. A review and consideration of the

rebuttal material in Record No. 086074 failed to satisfy concerns about the limitations of this study which continues to be considered inadequate for risk assessment purposes (G. Chernoff, 3/22/90).

228-035 000913 Earlier version of 001:019849, cited above. No worksheet. Aldous 4/22/91.

228-037 023535, also -028 945372 Earlier versions of 001:019851, cited above. No worksheet. Aldous 4/22/91.

077 086074, "Reply of Donald R. Taves, M.D., M.P.H., Ph.D. to comments in the "Toxicology Summary Report Worksheet" evaluating the University of Rochester Study of the fertility of workers exposed to Ordram", (D. Taves, 2/11/90). Rebuttal comments to the review in Record No. 019849-019852 (G. Chernoff, 3/23/90).

077 086075-086077, "Molinate: Industrial Summaries - Exposure Data", (Stauffer Chemical Co., 6/4/82). This volume contains summaries of exposure information from three molinate production facilities which were included in the epidemiologic study conducted by the University of Rochester. The data are helpful in clarifying the respiratory exposure levels associated with various jobs in the three plants. However, there is no indication of the nature of the jobs and which jobs may have included some dermal as well as respiratory exposure. Most importantly there is no indication of how many people of various job categories were included in the study population, or what the criteria for inclusion may have been (this review is contained in a memorandum from Dr. Michael O'Malley of Worker Health and Safety to G. Chernoff, dated April 2, 1990).

228-174 158168 Tomenson, J. and H. L. Northrop, "Report No. CTL/C/3097 - An assessment of fertility in male workers exposed to molinate at the Stauffer Chemical Company", Zeneca Central Toxicology Laboratory, Alderley Park, 7/14/95. A re-evaluation of the data originally reviewed as Record Nos. 019849-019852, above, incorporating the concerns of critics of the original submission. No DPR worksheet. Aldous, 6/30/98.

228-175 158169 "An assessment of fertility in male workers exposed to molinate at the Stauffer Chemical Company: Executive summary and peer review comments". Report No. CTL/C/3098. 11/10/97. This appears to be the study reviewed by Dr. Chernoff in 1990 under DPR Record Nos. 019849 to 019852, later re-worked by Zeneca, Inc. (see DPR Record No. 158168, above). The executive summary and the comments by four reviewers (Drs. Marsh, Matanoski, Savitz, and Checkoway) concluded that the data provided no evidence of a treatment effect of molinate on reproductive effects in workers. No worksheet. Aldous, 6/30/98.

228-136 120199 Paddle, G. M., "Epidemiological assessment of fertility in male workers exposed to 'Ordram' at the Stauffer Chemical Company", from a larger report with the same title by Taves et al., dated 4/20/84. Some information to partially characterize chemical plant worker exposure ranges is provided. Data include seasonal sperm counts, % normal sperm by season, and fertility analyses, with these data categorized in some cases by exposure groups. Seasonal data are provided because the primary exposure is seasonal (especially winter and spring). Data do not implicate molinate as a male

reproductive toxicant. Information is potentially useful, but not relevant for an SB-950 review worksheet. Aldous, 6/15/94.

228-187 171513 Tomenson, J. A. *et al.*, "An assessment of fertility in male workers exposed to molinate". This is a published report of the 1984 report generated by the University of Rochester in 1984 (DPR Document No. 228-001, Record Nos. 019849 to 019852). The present report has been examined by Dr. Cochran for possible incorporation in the Molinate risk assessment. Aldous, 12/30/99 (no worksheet)

TERATOLOGY, RAT{tc \l1 "TERATOLOGY, RAT}

****081 088187**, "A Teratology Study in CD7 Rats with R-4572 Technical", (Minor, J. L., Ciba-Geigy Environmental Health Center, Report # T-13266, March 30, 1990). R-4572 Technical, 97.6%, Lot #EHC-0866-30, was administered by gavage to groups of 26 female Crl:CD (SD) BRVAF/Plus rats on days 6-15 of gestation at dose levels of 0 (corn oil vehicle control), 2.2, 35, or 140 mg/kg/day. At 140 mg/kg/day, maternal food consumption and weight gain were reduced, and salivation was observed with the presence of cholinesterase inhibition. The number of resorptions at 140 mg/kg was dramatically increased, the litter size decreased, and evidence of intrauterine growth retardation present (decreased fetal weight, dilated brain ventricles, incomplete ossification of the sternbra). Maternal NOEL = 35 mg/ml/day (decreased food consumption and weight gain; salivation; cholinesterase inhibition); Developmental NOEL = 35 mg/kg/day (increased resorptions and intrauterine growth retardation). The study is **acceptable**, and a **possible adverse effect** (increased resorptions prior to the onset of maternal toxicity) is noted (G. Chernoff, 5/9/90).

003 035757. Not a true teratogenicity study, but is a spin-off from 3-generation reproduction study (003 945351). Delivered pups were examined instead of obtaining by C-section. Dosing of dams was ongoing, rather than optimized during organogenesis, as required for a teratogenicity study. **No adverse effects** reported by investigators. **Not acceptable** nor upgradeable. Review of full study (003 945351) by J. Christopher, 6/4/85.

TERATOLOGY, RABBIT{tc \l1 "TERATOLOGY, RABBIT}

****044 033591**, "A Teratology Study in New Zealand White Rabbits with Ordram [T-11866]", (Stauffer, CT, 6/6/85). Ordram technical, 98.8% purity; tested at 0, 2, 20, and 200 mg/kg/day by gavage. Apparent maternal and developmental toxicity NOEL = 20 mg/kg/day. The following observations in 200 mg/kg/day dams were statistically non-significant, although possibly treatment-related: increased abortions (incidence of 1, 1, 1, and 4 in increasing doses), slight decrease in body weight during days 14-21 of gestation (diminished food intake during same period), slight increase in liver weights and in incidence of dark brown liver (incidence of 1, 2, 2, and 5 in increasing doses). Fetuses in 200 mg/kg/day group had increased incidence of 5th unossified sternbrae and decreased incidence of extra ribs, (short, bilateral). **No adverse effects** noted: evidence for both

maternal and developmental toxicity initially evaluated as tenuous with study unacceptable but upgradeable with dose justification (J. Christopher, 9/17/85). Rebuttal in 228-050 (no record number) discusses the dose selection based on a range-finding study at doses of 200, 600 and 800 mg/kg/day with 5/group. Significant toxic effects were seen at 600 and 800 mg/kg/day supporting selection of 200 mg/kg as the high dose. The study is upgraded to **acceptable** status based on the justification of dose selection. (Aldous and Gee, 6/17/88).

228-135 119910 Wilczynski, S. L., "A range-finding teratology study in pregnant New Zealand White rabbits with ORDRAM*." Report No. T-11754, 12/20/83. This is a more complete report of the study earlier reported in Document No. 228-050 (see above 1-liner). Two of the 800 mg/kg/day dams died after a single dose, and treatment was terminated for that group after the single dose. The 600 mg/kg/day group suffered the first treatment-related death after 6 dose administrations, and treatment was terminated after the sixth dosing for that group. Only one pregnant, surviving doe was present at cesarean section for each of the 600 and 800 mg/kg/day groups: each had total litter resorptions. There was no evident general or reproductive toxicity at 200 mg/kg/day, hence this dose level was chosen for the definitive study. Aldous, 6/15/94 (no worksheet).

TERATOLOGY, MOUSE{tc \l1 "TERATOLOGY, MOUSE}

006 028493, "Ordram Safety Evaluation by Teratological Study in the Mouse", (Woodard Research Corp., 4/20/67). Ordram technical (96.5%). 0, 8, and 24 mg/kg/day in diets from day 6 to day 15 or 18. No maternal nor developmental toxicity indicated. **Unacceptable**, not upgradeable (too few pregnant dams/group, too few treatment groups, dosages not justified and apparently too low, etc.). (J. Christopher, 6/3/85).

GENE MUTATION{tc \l1 "GENE MUTATION}

General Comments: Six studies with microbial systems present a consistent picture of no mutagenicity with or without activation. However, an excellent study with mammalian cells demonstrated weak mutagenicity with activation. Thus there is a **possible adverse effect** for this category. All studies in this section (except for Record # 071077) have been reviewed again by Davis, 4/2/87, in making these conclusions. (Aldous, 4/7/87).

**063 071077, "Molinate: An Evaluation in the Salmonella Mutation Assay", (ICI Central Toxicology Laboratory, project no. CTL/P/2246, September 28, 1988). Molinate, purity 97.6%, two trials at 0 (DMSO), 1.6, 8.0, 40, 200, 1000, or 5000 µg/plate with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100, with and without Aroclor-1254 induced rat liver S9-mix, 64-68 hours incubation; tested a third time at 0.32, 0.8, 1.6, 4.0, 8.0, or 20.0 µg/plate with S9-mix activation on strains TA1535 and TA1537. No consistent increase in revertants. ACCEPTABLE. (Gee, 11/17/89)

006 945358, "Evaluation of Herbicides for Possible Mutagenic Properties", (K. J. Anderson et al., J. Agr. Food Chem. 20:649-656, 1972). Salmonella assay (Columbus Laboratories, Battelle Memorial Institute). Molinate stated to be negative for mutagenicity as tested with 8 mutants of Salmonella typhimurium. No adverse effect. Incomplete, **Unacceptable**. (Christopher 6/3/85).

006 945359, "Assay of Ordram for Mutagenicity Using the Ames Salmonella Tester System", (Woodard Research Corporation, 5/9/75). Ordram = molinate (purity unstated) tested with 0.1 ml of 0, 0.005, 0.5, and 50.0 ppm using strains TA 100, TA 1535, TA 1537, and TA 1538 with and without activation. **No adverse effects** reported. Incomplete, **Unacceptable**: no individual plate counts, inadequate positive controls, strain TA 98 not tested, too few dose levels, inadequate S-9 protocol, test material inadequately characterized. (Christopher, 6/3/85).

006 945360, "Mutagenic Evaluation of Compound Ordram Technical RCK 0701", (Litton Bionetics, Inc. 7/7/75). Ordram (purity unstated) tested at 6 dose levels ranging from 0.01 to 500 ul/plate using Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, and yeast strain D4, with and without activation. Toxicity was found at 100 and 500 ul/plate. **No adverse effects** reported. Incomplete, **Unacceptable**. Missing page 1, apparently only one plate for each dose level, negative control values with activation are high, test material inadequately characterized. (Christopher, 6/3/85).

006 945361, "Mutagenicity Testing on Molinate in Microbial Systems", (Institute of Environmental Toxicology, Japan 9/9/77). Molinate (99.8% purity) tested at 0, 10, 50, 100, 500, 1000, and 3000 ug/plate with Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, and Escherichia coli strain WP2 hcr with and without activation. 3000 ug/plate was toxic. **No adverse effects**. Incomplete, unacceptable, no test material lot number, no QA or sign-off sheet, only duplicate

plates, no confirmatory repeat assay. Study previously classified as acceptable by Christopher (6/3/85), now re-classified as **Unacceptable** by Davis (4/2/87) for reasons stated above.

228-006 035755 This appears to be a re-numbering of 006:945361 (two different record numbers were used for the two in vitro portion of the report. Aldous, 4/22/91.

006 035756, "Mutagenicity Testing on Molinate in Microbial Systems", (Institute of Environmental Toxicology, Japan 9/9/77). Molinate (99.8% purity) tested at 0, 30, or 100 mg/kg/dose in two doses by gastric intubation to 6 male mice per group, followed immediately by intraperitoneal inoculation with S. typhimurium G46 (his-). Three hours later peritoneal fluid was plated in triplicate for each animal and incubated for 2 days. A concurrent in vitro reverse mutation assay was done with the same strain using 0, 10, 50, 100, 500, 1000, and 3000 ug/plate. **No adverse effects**. Incomplete, **unacceptable**. No test material lot number, no QA or sign-off sheet, no evidence that the bacteria are exposed to molinate in the host-mediated assay. (Christopher, 3/85).

****043 026459**, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test, Forward Mutation Assay", (Stauffer Chemical Company, Report No. T-11840, 9/25/84). Ordram (= molinate) Technical (Lot No. WRC 4921-8-9, 98.8% purity) in a series of assays in L5178Y TK +/- cells with doses ranging from 0.0125 to 0.28 ul/ml without activation and from 0.01 to 0.10 ul/ml with activation, using 48 or 96 hour expression time. No mutagenicity was observed in the absence of activation, but activation with either mouse or rat liver S-9 extract induced marginal (2 to 5-fold) increases in mutation frequency. This was reproducible and not due to artifacts of selection or toxicity. **Possible adverse effect**, complete, **acceptable**. (Remsen (Gee), 9/11/85).

CHROMOSOME EFFECTS{tc \l1 "CHROMOSOME EFFECTS}

General Comment: It is noted that there were some elevated frequencies of both chromosome aberrations and sister chromatid exchanges with activation in Record 26460. This could support the weak mutagenicity with activation found in the same cell line in Record 26459. However, the cytogenetic effects were not consistent in repeat assays and were not dose-related, since none occurred in the high dose. Furthermore, the bone marrow micronucleus assay was negative. On balance the test material appears to be negative for this category of genotoxicity. We conclude that there is **No adverse effects**. The studies in this section (except for Record # 072638) have been reviewed again by Davis, 4/2/87, in making these conclusions.

The publication in Mutation Research, 1990, by Pinter et al. reports an increase in the incidence of micronucleated polychromatic erythrocytes. The report is unacceptable based primarily on the lack of individual data. There is already an acceptable study of this type spanning the same dose range without an effect. Therefore, there is no demonstrable adverse effect. Added comment by Gee, 8/11/94.

Note: A dominant lethal study, Record No. 146938, has been reviewed as negative for dominant lethality. Gee, 1/16/97.

**065 072638, "Molinate: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes", (ICI Central Toxicology Laboratory, Report no. CTL/P/2402, Dec. 15, 1988). Molinate, purity 97.6%, at levels of 0 (DMSO), 190, 95 and 24 µg/ml with lymphocytes of 2 donors (1 male and 1 female), in vitro - 3.25 to 3.75 hours exposure, in the presence and absence of Aroclor-1254 induced male rat liver activation. No adverse effect. Molinate showed no clastogenic effect with human lymphocytes in vitro. ACCEPTABLE. (Kishiyama, 7/21/89 and Gee, 11/17/89).

043 026461, "Mutagenicity Evaluation in Bone Marrow Micronucleus", (Stauffer Chemical Company, Report No. T-11820, 11/22/83). Ordram (molinate) Technical (Lot No. WRC 4921-8-9, 98.8% purity) at 0, 200, 400, or 600 mg/kg for 15 male mice/group and 0, 100, 200, or 400 mg/kg for 15 female mice/group by single oral gavage. Sampled 5000 bone marrow cells of 5 mice/sex/time point at 24, 48, and 72 hours (except for 24 hour female group where sampled 10,000 cells). **No adverse effects, Complete, **acceptable**. [Remsen (Gee), 9/11/85].

043 026460, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test. Cytogenetic Assay." (Stauffer Chemical Company, Report No. T-11856, 12/2/83). Ordram (molinate) Technical (Lot No. WRC 4921-8-9, 98.8% purity) in a series of assays in L5178Y mouse lymphoma cells with doses ranging from 0.0125 to 0.20 ul/ml without activation and from 0.0025 to 0.0400 ul/ml with activation. Neither chromosome aberration frequency nor sister chromatid exchange (SCE) frequency was increased in the absence of activation. Activation with rat liver S-9 extract resulted in some statistically significant elevations in both chromosome aberration and SCE frequencies but these were not dose-related and not repeatable. **No adverse effects, complete, **acceptable**. (Remsen, 9/11/85).

No record number "Cytogenetic effect of the thiocarbamate herbicides butylate, molinate and vernolate in the mouse bone marrow micronucleus test." Pinter, A. et al. in: Mutation Research 242: 279 - 283 (1990) Molinate, 97.4%, was given to CFLP mice in a single oral dose at 0 (sunflower oil), 175, 350 or 525 mg/kg. Groups of 5/sex (usually) were sacrificed at 24, 48 and 72 hours for control and high dose and at 48 hours at the low and mid doses. Micronuclei in polychromatic erythrocytes were scored, 1000 per animal. AT 48 hours at the mid and high doses, the mean incidence of micronucleated polychromatic erythrocytes was statistically significantly increased in both males and females compared with controls. **Possible adverse effect. Unacceptable** (no individual data, missing information on study conduct). (Gee, 8/11/94).

** 228-155 146938 "Molinate: Dominant lethal study in the rat" (M. E. Moxon, Zeneca, Central Toxicology Laboratory, UK, Report no. CTL/P/4778, Study no. RR0675/MN, 12/18/95) Twenty male rats (Sprague Dawley Crl: CD(SD)BR) per group were treated with molinate technical (batch P4 D7534/25, 96.8%) at 0 (diet), 5, 10 or 15 ppm. The males were part of a multigenerational reproduction study and had received molinate for 12 weeks before being transferred to the dominant lethal study. Two additional weeks of treatment were followed by mating 1 male: 2 females for 7 overnights followed by a second series of females for 7 overnights. The animals were not fed during the overnight mating period but were fed during the day. Females were killed on day 20 after the first overnight and evaluated for corpora lutea, live implantations and early and late intra-uterine deaths. Methylmethane sulfonate (MMS) at 40 mg/kg by a single intraperitoneal injection was the positive

control. No dominant lethality was reported with molinate. Male fertility, however, was affected at 15 ppm. MMS was positive with marked increases in early intra-uterine death. The study is acceptable with no adverse effect reported. (Gee, 1/15/97)

DNA DAMAGE{tc \l1 "DNA DAMAGE}

General Comment: the evidence shows **no adverse effects** in this category. See also the general comments for the Chromosome Mutation category and the one-liner for Record 26460. The studies in this section (except Record # 073902) have been reviewed again by Davis, 4/2/87 in making these conclusions.

**068 073902, "Molinate: Assessment for the Induction of Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures", (ICI Central Toxicology Laboratory, Study no. SV0332, Report No. CTL/P/2484, March 22, 1989). Rat hepatocytes were exposed to molinate, purity 97.6%, at concentrations of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , or 10^{-9} molar for 17 to 20 hours in 2 independent tests. Doses of 10^{-4} and 10^{-5} were selected as the high concentration for experiments 1 and 2, respectively, and the three subsequent lower concentrations from each experiment were also were selected for UDS evaluation. Net nuclear grain counts were less than zero for all molinate treatments examined. No adverse effects indicated. ACCEPTABLE. (Kishiyama and Gee, 11/17/89).

006 945361, "Mutagenicity Testing on Molinate in Microbial Systems", (Institute of Environmental Toxicology, Japan 9/9/77). Molinate (99.8% purity) tested at 0, 1, 5, 10, 25, 50, and 100% v/v on the paired *B. subtilis* strains H17 (repair competent) and M45 (repair deficient) in a disc diffusion assay using the streak technique. **No adverse effects. Complete, **acceptable**. (Christopher, 6/3/85).

NEUROTOXICITY, HEN{tc \l1 "NEUROTOXICITY, HEN}

046 041548 Study T-10510, "Acute Delayed Neurotoxicity Study with Ordram Technical in adult hens", (Stauffer, 6/16/83). [T-10510]. Molinate, technical. Dosages (see section V. A. of this review for details) 0.02, 0.063, 0.20, 0.63, and 2.0 g/kg + positive and negative controls. Additional hens for recovery study. NOEL for neurotoxicity (apparently not identical to classical TOCP-like acute delayed toxicity) = 0.2 g/kg, based on microscopic lesions (especially axonal degeneration in upper spinal cord and cerebellar peduncle areas), walking behavior deficits, and other observations of neuromuscular weakness in 2.0 and 0.63 g/kg hens. Mortality, weight deficits, and other general toxicity findings were dose-related and essentially limited to the same upper two dosage groups. **Not acceptable**, but fully upgradeable on receipt of additional clinical observation data. (C. Aldous, Aug. 7, 1986).

NEUROTOXICITY, RAT{tc \l1 "NEUROTOXICITY, RAT}

****228-147 129725** Horner, J. M., "Molinate: Acute neurotoxicity study in rats", Zeneca Central Toxicology Laboratory, Alderley Park, Report No. CTL/P/4180, 3/22/94. Twelve Alpk:APfSD rats/sex were dosed once with 0, 25, 100, or 350 mg/kg by gavage. Rats were evaluated for limited Functional Observational Battery (FOB) parameters, motor activity, cholinesterase effects, and histopathology of nervous system. No NOEL was found for the following acute effects: food consumption and body weight decrements (M), sensory response (tail flick) (M & F), and motor activity (M & F). All of the above had dose-response relationships. Brain cholinesterase NOEL at day 15 = 25 mg/kg (dose-related changes in M & F). There were no effects which persisted for more than a few days after a single dose of 25 mg/kg, which is the NOEL for non-transient effects (based on slight brain weight decrements in males). A **""possible adverse effect""** is indicated, based on neuronal cell necrosis in the pyriform cortex of 350 mg/kg females. The resulting NOAEL = 100 mg/kg. The study was originally classified as unacceptable due to deficiencies in the reporting of the FOB data. Study was upgraded to **acceptable**, based on the report revision in Record 157794 (see below). Deficiencies which do not invalidate the study are (1) some groups were too small for meaningful evaluation, such as N = 3 for glial fibrillary acidic protein (GFAP) assays, and (2) there were no concurrent positive controls (however these were supplied by Zeneca in support of another active ingredient). Aldous; 6/9/94, 6/9/98 and 6/23/98. NOTE: The re-reading of the pyriform cortex and to the ventral portion of the hippocampal dentate gyrus described below found only one high dose female with treatment-attributable neuronal cell death in the pyriform cortex. This is a reduction of the numbers of rats affected, but does not change the NOEL for this lesion type.

228-165 157794 Horner, J. M., "First revision to molinate: Acute neurotoxicity study in rats", Addendum to Document # 228-147, Record # 129725. Date of report revision: 11/22/95. The essential contribution of this report is assurance that the essential parameters of an FOB were evaluated. Although the FOB observations were presented without graded responses, the FOB investigators (who were "blind" as to treatment group of test rats) clearly demonstrated the ability to identify a coherent symptomatology attributable to treatment. The study is upgraded to **acceptable**. Aldous, 6/23/98.

228-163 157792 Chalmers, D. T., S. J. Duffell, and S. A. Horner, "Thiocarbamates: Selective re-examination of neuropathology", Zeneca Central Toxicology Laboratory, Alderley Park, 3/28/95, Laboratory Report # CTL/P/4618. This is a consensus report presented by two pathologists, who re-evaluated rat brain slides relating to acute and subchronic neurotoxicity studies on six thiocarbamate compounds, including cycloate, EPTC, molinate, pebulate, vernolate, and butylate, plus a 21-day inhalation neurotoxicity test using cycloate. A major objective of this study was to identify treatment-related cell death, as distinguished from occasional dead cells reflecting remodeling processes of normal development. Re-examinations were limited to pyriform cortex and to the ventral portion of the dentate gyrus: areas known to be uniquely susceptible to thiocarbamate neurotoxicity. Results were as follows for acute neurotoxicity testing [values in parentheses are (NOEL, LOEL)]: cycloate (no NOEL, 200 mg/kg), EPTC (200, 1000 mg/kg), molinate (100, 350 mg/kg), pebulate (150, 500 mg/kg), vernolate (150, 500 mg/kg), and butylate (600, 2000 mg/kg). Results for subchronic testing were: cycloate (40, 400 ppm), EPTC (500, 2500 ppm); other compounds negative for subchronic testing, with HDT's of molinate (450 ppm), pebulate (1000 ppm), vernolate (1000 ppm), and butylate (5000 ppm). In addition, cycloate was tested in a 21-day inhalation study, yielding NOEL = 1.2 µg/l and LOEL = 12.0 µg/l. Useful supplemental data to clarify study results. Aldous, 6/9/98.

228-171 157802 Horner, S. A., and Duffell, S. J., "Morphometric evaluation of the developing rat brain", Zeneca Central Toxicology Laboratory, Alderley Park Report No. CTL/P/5089. Study was to obtain reference information on brain weight, length, width, and various morphometric measurements in the course of growth and development. Alpk:APfSD rats (6/sex/sacrifice interval) were killed at postnatal days 7, 10, 12, 14, 16, 22, 29, 42, or 63. Reference data are useful for evaluation of acute and subchronic studies on molinate. For example, the control brain weights at about day 56 in Record No. 129725, above, were just under mean weights obtained in this evaluation for postnatal day 63. Useful reference data, no worksheet. Aldous, 6/23/98.

****228-148 130928** Horner, J. M., "Molinate: Subchronic neurotoxicity study in rats", Zeneca Central Toxicology Laboratory, Cheshire, UK, 10 May 1994, Study No. PR0949. Alpk:APfSD rats (12/sex/group) were dosed in diet for at least 90 days with 0, 50, 150, or 450 ppm molinate, purity 96.8%. Motor activity and limited functional observational battery (FOB) measures were recorded pretest and at weeks 5, 9, and 14. Gross and microscopic examinations of nervous system tissues were conducted at termination. NOEL for structural/functional nervous system changes = 150 ppm (slight decrease in brain weight in both sexes; increase in sciatic nerve degeneration in males). No NOEL exists for inhibition of enzyme biomarkers associated with neurological changes (brain neuropathy target esterase (NTE) inhibition in both sexes at all dose levels; brain acetylcholinesterase (AChE) inhibition in females at all dose levels and in males at 150 to 450 ppm). No NOEL exists for non-neurological effects (modest body weight decrements at 50 and 150 ppm in females, associated with very modest decrements in food consumption). High dose males and females had more definitive BW and FC decrements. The study indicates a **"possible adverse effect"**, based on brain weight decrements at the high dose, on brain AChE and NTE activity decrements over a range of dose levels, and on indications of treatment-related sciatic nerve degeneration. Study was first classified as unacceptable due to deficiencies in the design and conduct of the FOB. Following review of Record No. 157793 (below), study has been re-classified as acceptable. Aldous, 5/10/95, 6/26/98.

228-164 157793 Horner, J. M., "First revision to molinate: Subchronic neurotoxicity study in rats"; addendum to Document # 228-148, Record # 130928. Date of report revision: 12/14/95. Laboratory Report No. CTL/P/4289. The essential contribution of this report is assurance that the essential parameters of an FOB were evaluated. Although the FOB observations were presented without graded responses, the FOB investigator assigned to this study (who was "blind" as to treatment group of test rats) was sufficiently trained to effectively identify any coherent symptomatology attributable to treatment. The study is upgraded to acceptable, with no change in DPR interpretation of the outcomes of this study. Aldous, 6/26/98.

DEVELOPMENTAL NEUROTOXICITY, RATS{tc \l1 "DEVELOPMENTAL NEUROTOXICITY, RATS}

****228-166 157797** Horner, S. A., "Molinate: Developmental neurotoxicity study in rats", Zeneca Central Toxicology Laboratory, Alderley Park, 6/13/96, Laboratory Report # CTL/P/4994. Technical molinate, 96.8% purity, was administered to 30 Alpk:AP₁SD dams/group at 0, 20, 75, or 300 ppm from gestation day 7 through lactation day 11. Developmental neurotoxicity evaluations were performed on offspring, typically shortly after cessation of treatment of dams and at day 63. Tests included motor activity, learning and memory testing (water maze), auditory startle tests, histopathology (restricted to brains at day 12 following immersion fixation: expanded to various central and peripheral nervous system structures on day 63 following perfusion fixation), and morphometric analyses of brain transverse sections at 7 levels. Pup weights, clinical signs, and appearances of developmental landmarks (preputial separation and vaginal opening) were monitored. Apparent maternal NOEL = 75 ppm (body weight and food consumption decrements during gestation and lactation). Developmental NOEL = 20 ppm (reduced thickness of the molecular layer of the prepyramidal fissure of the cerebellum at day 12). Prominent findings in F1 rats included increased pup mortality during maternal exposure period, slightly delayed time to preputial separation or vaginal opening, and slight delays in learning and memory skills (only in tests conducted within 2 weeks of maternal exposure). In addition, some changes persisted to the end of the study (measurements on days 61 to 63), including decreased amplitude of response to auditory stimuli and/or increased delay to maximum amplitude of response, decreased brain weight, and reduced body weight. Small group sizes and large intra-group variability limited the usefulness of brain morphometric analyses. There were no histopathology changes at day 12 or day 63. Brain weight effects and marked but transient morphometric cerebellar changes at the LEL are **possible adverse effects**. **Acceptable study**. Aldous, 7/8/98.

228-151 132852 Allen, S. L. "Molinate: Preliminary developmental neurotoxicity studies in Alpk:AP₁SD rats", Zeneca Central Toxicology Laboratory, Cheshire, UK, 8/26/94. Ten females/group were dosed by gavage with 4, 8, 15, 35, or 50 mg/kg/day molinate from gestation day 7 until lactation day 11 to set dose levels for a definitive rat developmental neurotoxicity study. Study was done in two segments, with 10 controls and ten 15 mg/kg/day dams per segment. The highest dose level without developmental toxicity was 4 mg/kg/day, based on pup mortality at 8 mg/kg/day and above (3 total litter losses at 8 mg/kg/day). Maternal toxicity NOEL = 8 mg/kg/day, based on body weight and food consumption decrements. Fifty mg/kg/day caused a marked drop in gestational survival and a slight delay in gestation time. This study justifies 4 mg/kg/day as the high dose for the definitive study. No DPR worksheet. Aldous, 5/5/95.

228-156 147179 Allen, S. L., "Molinate: Preliminary dietary developmental neurotoxicity study in rats", Central Toxicology Laboratory, Cheshire, UK, 3/26/96. Report No. CTL/P/4946. Ten Alpk:AP₁SD dams were dosed in diet with 0, 300, 500, or 800 ppm molinate from p.c. day 7 to lactation day 11. Study was to set dose levels for a developmental neurotoxicity study. Dam body weights were reduced in dose-related fashion (statistically significant at 500 ppm upward). Food consumption was significantly reduced for all groups: dose-related. All 800 ppm pups died by lactation day 11. Nearly half of 300 and 500 ppm pups also died or were missing (presumed cannibalized) by this time, following dose-related pup body weight decrements. Investigators judged that 300 ppm is the highest dose level suitable for the definitive developmental neurotoxicity study (a valid assessment). Aldous, 7/11/96.

228-169 157800 Allen, S. L., "Developmental neurotoxicity study in the rat using diet restriction", Central Toxicology Laboratory, Cheshire, UK, 12/1/95. Report No. CTL/P/4383. Groups of 30 timed-mated Alpk: AP_{SD} dams were subjected to 4 dietary regimens from p.c. day 7 to lactation day 11: (1) *ad lib.* access to food, (2) *ad lib.* access to food, with the daily additional gavage administration of 10 ml/kg distilled water, (3) *ad lib.* access to food for only 6 hr per day, and (4) dietary restriction to 22 g diet/day during gestation and 32 g diet/day during lactation. Observations in F1 offspring included motor activity, learning and memory tests (water maze), auditory startle, appearance of developmental landmarks (vaginal opening and preputial separation), clinical observations, and histopathology (methods as in Record # 157797, below). There were no distinguishable differences in outcomes from groups 1 and 2. Maternal body weights in group 3 and 4 dams were reduced about 50 g during gestation, compared to groups 1 and 2; similar decrements continued in groups 3 and 4 during lactation, with partial recovery after diet restriction ended. Pup weights did not vary between groups on day 5, but group 3 and 4 pups fell behind in body weight by day 12. This body weight decrement was transient in F1 females, but a small but significant decrement persisted in F1 males through day 63. There were otherwise no indications of developmental toxicity in offspring of diet-restricted dams. This study provides reference data for comparison with molinate studies such as Record # 157797. No worksheet. Aldous, 3/25/98.

228-170 157801 Allen, S. L., "Trimethyltin chloride: Investigation of neurotoxicity in rat pups using morphometrics and startle response", Zeneca Central Toxicology Laboratory, 6/21/96, Laboratory Report # CTL/P/5043. The test data are consistent with results of published studies on trimethyltin, and attest to the ability of the test facility in assessing startle response, brain histopathology, and brain morphometric measurements. Representative results are included in the DPR review. Aldous, 6/8/98.

228-168 157799 Allen, S. L., "Assessment of learning and memory in rats", Zeneca Central Toxicology Laboratory, Alderley Park, 4/5/94. To validate the use of the Y-shaped water maze used in developmental toxicology studies, 3 groups of 20 rats/sex were dosed ip just before the learning phase (day 1) or just before the memory test phase (day 4) as follows: (1) with vehicle (demonized water, 10 ml/kg) on both days, (2) scopolamine HCl (10 mg/kg) on day 1 only, or (3) scopolamine HCl on day 4 only. All rats were placed in the Y-maze 10 times on each test day, recording the time to escape each time. Also, each rat was timed while swimming a straight channel of comparable length just after the series of maze events. The expected outcome was that scopolamine would have no effect on performance on the dosing day, but would affect the rats' ability to recall experience from the learning session when tested subsequently in the "memory test" phase (impaired "reference memory"). Reference memory assessed on day 4 was impaired in both sexes among rats dosed on day 1 (i.e. maze completion took longer on the first 1 or 2 trials on day 4 compared to controls). Swimming ability was unimpaired, and there were no consistent unexpected differences. This study validates the technique for developmental toxicology assessment (no DPR worksheet). Aldous, 6/30/98.

228-167 157798 Allen, S. L., "Measurement of motor activity in rat pups", Zeneca Central Toxicology Laboratory, Alderley Park, 12/7/93. Report No. CTL/P/4155. This was a validation study for motor activity assessment in rat pups of age 14 to 22 days. Positive controls were 0.5 mg/kg amphetamine sulfate and chlorpromazine HCl. Amphetamine elicited some stimulatory effects in males at all sessions, and in females at day 22. Chlorpromazine generally reduced activity in day 14 and 18

females and probably also on day 22 males. It appears that equipment was working satisfactorily, however data show practical limitations of assessing motor activity responses in young pups, since classical responses obtainable in more mature rats could not reliably be obtained in young rats. Data do not invalidate the developmental neurotoxicity study, but demonstrate practical limitations of this component. No DPR worksheet. Aldous, 6/30/98.

METABOLISM STUDIES{tc \l1 "METABOLISM STUDIES}

NOTE: Metabolism studies are not routinely evaluated under SB-950, hence the 1-liners below do not represent an exhaustive listing of such studies which may be available. In particular, there are rat metabolism studies cited in a record dated 11/30/92 entitled "Molinate: NOEL's being used in CDPR risk assessment" (DPR Record No. 119319) which suggest a metabolite pattern in rats which is markedly different from man. Humans excrete molinate metabolites almost entirely in the urine, and 4-hydroxymolinate is by far the major metabolite. Rats apparently excrete more molinate mercapturate conjugate than 4-hydroxymolinate, and an appreciable amount of rat metabolites are found in feces. Aldous, 6/16/94.

074 090135, ICI America Inc., 12/20/89. This report consists of 4 tables from a draft report on a dermal absorption study conducted as part of a research project that did not use the "Zendzian" protocol. An additional in vitro dermal penetration study, followed by an in vivo (Zendzian protocol) study are scheduled with tentative reporting dates of 4/1/90 and 12/31/90, respectively. No worksheet provided at this time (G. Chernoff, 1/25/90).

228-127 118234 Batten, P. L. et al., "Molinate: Metabolism in man following a single oral dose", Report No. CTL/R/1099, March 6, 1992. Report was submitted in support of DPR risk assessment process. Oral administration of 5 mg/person molinate in corn oil led to rapid urinary excretion. Principal metabolites were conjugates of 4-hydroxy molinate (about 39% of administered dose). It was suggested that this metabolite could be used to estimate absorbed dose. By comparison, only about 1% of the dose was excreted as molinate mercapturate. This study cited unpublished results of Krieger *et al.*, who determined that doses of 0.025, 0.05, 0.1, 0.6, and 0.7 mg/kg molinate in humans yielded following respective amounts of mercapturate as percent dose recovered in urine: 1.1, 1.6, 1.8, 5.5, and 6.2%. The latter result suggests that at dose levels likely to be encountered by humans, the amount of metabolism via oxidation of sulfur would be small. No DPR worksheet by DPR SB-950 Data Review Group (not a required study type). Aldous, 6/15/94 and 9/1/00.

228-132 118003 Lythgoe, R. E. et al., "Molinate: Excretion and blood kinetics in the monkey". Ring-labeled molinate was administered iv or orally to male cynomolgus monkeys. Excretion was rapid, and almost entirely via urine. Animals were not sacrificed for general tissue analyses, however levels in RBC's and plasma dropped quickly. Major urinary metabolites following a 60 mg/kg oral dose were: glucuronide conjugate of 4-hydroxymolinate (33%), cysteine conjugate (12%), and molinate mercapturate (10%). [Contrast with rat metabolism in Record No. 118234, above]. Useful information, but not requiring SB-950 worksheet at this time. Aldous, 6/15/94.

228-179 158958 Lloyd, S. C., "Species comparison in the metabolism of the herbicide molinate", Zeneca Central Toxicology Laboratory, Alderley Park, 12/16/97. Report No. CTL/R/1355. Male animals were used: Sprague-Dawley rats, CD-1 mice, NZW rabbits, and beagle dogs. The primary labeled material was [^{14}C]-molinate (S-ethyl hexahydro-1H-azepine-2- [^{14}C]-1-carbothiolate). Report describes preparation of metabolite standards and MS and NMR peaks used in analyses. Major initial metabolism steps included sulfur oxidation, hydroxylation of the ring structure, or thiocarbamate cleavage. Investigators identified plausible routes for additional metabolic processes, including conjugation steps. No unconjugated sulfoxide nor sulfone of molinate were identified. These were important presumed intermediates, since the subsequent glutathione-derived conjugates were observed, primarily in the rat. The presence of oxidized sulfur products is an important feature of other studies (see report 228-178:158172). Valid supplemental data. Aldous, 6/25/98.

228-182 162795 Macpherson, D., "Molinate: Biotransformation in the cynomolgus monkey following single oral administration", Central Toxicology Laboratory (CTL), Cheshire, UK, 6/26/98. Report No. CTL/P/5923. Urinary and fecal samples were collected at Inveresk Research following single oral doses of 0.1, 2, or 40 mg/kg ^{14}C -molinate to 3 male monkeys (the same monkeys were used sequentially for each dose level, "with a suitable washout period between each phase"). Analyses of the samples were done at CTL. Excretion patterns were similar for each dose level, with over 80% of the dose recovered within 48 hr. Urine contained 68 to 77% of recovered label, compared to no more than 9% in feces. Virtually all fecal recovery was molinate. The most commonly encountered urinary metabolite was 4-hydroxy molinate glucuronide (25-26% of dose). An additional 6% was a hydroxy molinate glucuronide, for which the position of the hydroxyl could not be established. Maximum amounts (as percent of dose) measured for other identified metabolites were: molinate mercapturate (13.9%), a hydroxy molinate mercapturate (2%), molinate acid (the distal carbon of the S-ethyl group was oxidized to a carboxylic acid: 5%), molinate cysteine conjugate (6%), and a metabolite most likely to be the S-glucuronide (5%). An additional 4 highly polar metabolites constituted 3 to 8% of dose each, but could not be identified. The glutathione-derived metabolites, principally molinate mercapturate, constituted higher percentages of dose following higher two dose levels than at the 0.1 mg/kg level. Useful information, but not requiring SB-950 worksheet at this time. Aldous, 11/19/98.

228-189 176747 Lovatt, C., "Molinate: metabolism by sulfoxidation in the rat," Central Toxicology Laboratory, Alderley Park, 7/18/00. Report No. CTL/00A122. Male SD rats (aged 6-8 weeks), were dosed with ^{14}C -labeled molinate (96.9%) by gavage (4 mg/kg, corn oil vehicle) at 1, 16, 40, and 200 mg/kg (respective group sizes of 3, 1, 1, and 4 rats), then killed after collection of urine for 72 hours in metabolism cages. Only samples collected during the first 24 hr were analyzed, since most collected radioactivity was obtained during this interval. Several metabolites were analyzed, but only molinate mercapturate and the hydroxymercapturate were presented in this brief report. These were the major products of sulfur oxidation, relevant because other studies have implicated molinate sulfoxide as the primary reproductive toxicant. Mean amounts of these two metabolites (primarily the mercapturate), based on percent of urinary metabolites obtained in 24 hr, were 18, 24, 26, and 29% for lowest to highest dose levels, respectively. Investigators considered this to (1) confirm the high level of sulfur oxidation in the rat compared to man (see Document/Record No. 228-127 118234), and (2) suggest that the amount of sulfur oxidation in rats increases with increasing dose level. Group sizes

appeared too small to make a strong case for the latter conclusion. Useful supplemental information. Aldous, 9/6/00.

RISK ASSESSMENT/PROPOSITION 65 GENERAL SUBMISSIONS{tc \l1 "RISK ASSESSMENT/PROPOSITION 65 GENERAL SUBMISSIONS}

228-180 162789 Foster, J. R., and M. K. Ellis, "Molinate: A review of reproductive toxicity", Laboratory Project No. CTL/R/1376. Report does not present new data. Review article is similar in content to several others: no reviewable data, hence no worksheet. Aldous, 11/18/98.

228-136 120196 "Submission to January 1993 SAP: Molinate reproductive toxicology executive summary", ICI Agrochemicals, 12/21/92. A discussion referring primarily to SB-950-related studies, presenting several lines of evidence that reproductive effects in rat reproduction studies are species-specific phenomena. Information is potentially useful, but not relevant for an SB-950 review worksheet. Aldous, 6/15/94.

228-129 118021 "Molinate: A review of reprotoxicity" (Prepared by ICI Agrochemicals for submission to the California Proposition 65 Scientific Advisory Panel), 9/30/92. General discussion of reasons why the writers feel that molinate should not be classified by California Proposition 65 SAP as a reproductive toxin. Primary thrust of arguments is that high quality human epidemiological studies have been carried out, and no evidence of human reproductive toxicity has been found. No formal review or worksheet is relevant by this group at this time. Aldous, 6/16/94.

228-178 158172 Wickramaratne, G. A., "Report No. CTL/R/1335 - Molinate: Rodent reproductive toxicity and its relevance to humans: a review", 11/3/97. This is one of several brief reviews indicating that reproductive changes in rats do not indicate human risk potential. No unique data are found in this review. No DPR worksheet. Aldous, 6/30/98.

228-183 164252 Wickramaratne, G. A. de S., J. R. Foster, M. K. Ellis, and J. A. Tomenson, *Regul. Toxicol. Pharmacol.* **27**, 112-118 (1998). "Molinate: Rodent reproductive toxicity and its relevance to humans--A review". This is the publication of Record No. 158172, above. No DPR worksheet. Aldous, 11/17/98.

OTHER

066 072779, "21-Day Dermal Toxicity to the Rat", (ICI Toxicology Laboratory, Report No. CTL/p/2321, 1/27/89). Molinate, purity 97.6%, was administered undiluted to the dorso-lumbar region of 5 Wistar-derived albino rats/sex/group at concentrations of 0 (occlusive bandages only), 10, 25 or 50 mg/kg for 6 hours per day for 21 days. An increased incidence and severity of skin irritation (desquamation, thickening of skin, erythema, and oedema), and slight to moderate hydronephrosis was observed at doses greater than 10 mg/kg/day. NOEL = 10 mg/kg/day based on skin irritation and

hydronephrosis. This study is ACCEPTABLE as a supplemental report (J. Kishiyama and G. Chernoff, 1/25/90).